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A CYTOLOGICAL STUDY OF WATER-SOLUBLE AND FAT-SOLUBLE CONSTITUENTS OF CITRUS[1]

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INTRODUCTION

The objects of this cytological study of water-soluble and fat-soluble constituents of citrus were to get a clearer notion of the biochemical changes taking place in the normal citrus tree and resulting in the production of highly attractive fruit, and to learn what undesirable biochemical phenomena are associated with various pathological conditions or blemishes. To obtain these objects it was necessary to study the cells of both normal and pathological tissues; first, in the living condition, using vital dyes; secondly, in the postvital condition; and thirdly, after killing with proper killing fluids.

TECHNIC

STUDY OF LIVING CITRUS CELLS

If pieces of tissues are torn, scraped, or cut from living leaves, shoots, or fruits of citrus and immediately immersed in a sugar solution of the same molecular concentration as the cell sap, the cells that are not broken open remain alive for a few hours and show, when examined under high-power objectives, structures that may be considered very similar to the structures of the cells in the undisturbed tissues.

If a proper quantity of a vital dye, such as neutral red, is added to the sugar solution, the cell vacuoles will stain red.[3] The vacuoles are surrounded by the cytoplasm, which in the living cell is a hyaline mass, containing various more highly refractive bodies, such as fat droplets, essential-oil droplets, rodlike or threadlike mitochondria, and colorless or green plastids. Some of the plastids contain starch grains.

POSTVITAL STAINING

Whereas only some of the water-soluble compounds in the vacuoles may be demonstrated in the living cell by the use of vital dyes, fat bodies may be demonstrated by transferring the pieces of living tissues

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[2] The writer takes pleasure in acknowledging with thanks the assistance given by H. R. Fulton in suggesting problems and in helping in the preparation of this paper; by T. Ralph Robinson, H. E. Stevens, and J. F. Wootten, all of the U. S. Bureau of Plant Industry, and W. W. Yothers, of the U. S. Bureau of Entomology, in securing and identifying material; and by C. B. Bouis, president of the Florida Fruit Co., in making available the resources of his groves.

[3] Details regarding the staining technic used in these studies will be found in a previous article by the writer: DUFRENOY, J. STAINING CELL CONSTITUENTS IN DISEASED PLANT TISSUES. Stain Technol. 3: 57-63, illus. 1928.

into a solution of indophenol blue. This solution is toxic to the living cells and kills them within a few minutes, but a sufficient time is available for observations, during which vacuoles retain the red stain and the fat bodies or essential-oil drops are stained blue.

STUDY OF KILLED AND FIXED TISSUES

Vacuoles, which are so easy to demonstrate in the living cell by using vital dyes, are more difficult to demonstrate in killed tissues. The vacuolar material, which is in a colloidal state in the living cells, is easily thrown out of the colloidal state by the electrolytes in the killing fluid. Even the presence of such colloids as neutral red sometimes precipitates some of the vacuolar material into granular bodies alongside the cytoplasm at the margin of the vacuole. Such an effect is more likely to result from the effect of the killing fluid.

Great care must be taken not to mistake vacuolar precipitates for cytoplasmic inclusions. Most vacuolar precipitates are made of tannic material or phenolic compounds. Their staining reactions are somewhat similar to those of either fat bodies or oleoresins. For instance, osmic acid stains the phenolic precipitates a deep gray or solid black and fat bodies or essential-oil drops a more or less deep gray. Potassium bichromate when associated with formalin in the Regaud killing fluid generally preserves the tannoid contents of the vacuole in the form of a foamy yellow mass, or precipitates the vacuolar material into a number of small yellow drops. Tissues killed with the Regaud fluid display in yellow color about the same pattern as is obtained in red color when the vacuolar system is stained by neutral red in the living cell.

CYTOLOGICAL STUDIES

CYTOLOGICAL EVIDENCE OF WATER-SOLUBLE AND FAT-SOLUBLE CONSTITUENTS IN NORMAL AND PATHOLOGICAL CITRUS TISSUES

In any living cell, the metabolic products fall within one of the two following classes: (1) Water-soluble compounds, part of which are imbibed by the cytoplasmic colloids and part of which separate out of the cytoplasm and go into solution in vacuoles hollowed out of the cytoplasm; and (2) fat and fat-soluble compounds, combined more or less with proteins as part of the cytoplasm, or as mitochondria and plastids, or absorbed by suberized or cutinized cell walls, or existing free as fat droplets. In the case of citrus, oleoresins are produced in the form of droplets, which no ordinary microchemical test can differentiate from fat droplets.

As a rule, notions as to the aspect of any constitutent in the cell are to be obtained only by microscopic examination of the living cell in an isotonic solution with or without the use of vital dyes, or of sections from tissues killed by methods that best preserve both the microchemical and histological conditions prevailing in the living material.

Whenever pigments are formed in the cells they are either water soluble (anthocyans, which go into solution into the vacuolar contents) or fat soluble (chlorophyll, xanthophyll, and various pigments coloring plastids).

Citrus tissues are conspicuously lacking in anthocyan pigments, and their important pigments are fat soluble. These, besides the chlorophyll and the xanthophyll pigments to be found in any green

plants, are the orange pigments that give to the mature citrus fruits their desirable appearance.

As the vacuolar material never develops color under natural conditions, little attention has been given to the study of vacuoles in citrus. It should not be forgotten, however, that in these plants, more perhaps than in any other, not only does the vacuolar material form most of the valuable marketable part of the fruit, the juice, but also it forms the bulk of the vegetative parts. Huge vacuoles occupy most of the volume of the cells in the medullary rays, in the wood and phloem parenchyma, in the chlorophyllous tissues, and in the epidermis. Under natural healthy conditions the vacuolar contents of most of the cells are easily stained by vital dyes.

The first symptom of pathological conditions is made evident cither by a browning of the vacuolar contents or by a change in the response toward vital dyes. While the fact that the vacuolar content can not be stained is not proof that the cell is no longer living, the ability to stain the vacuolar contents with vital dyes brings definite proof of the living condition of the cell. Besides, the morphological aspects or patterns assumed by the vacuolar material in the cell can yield some useful indications as to the physiological condition of the cell.

The second symptom of a pathological or an abnormal condition in the cell is made evident by the swelling and vesiculation of the lipoprotein complexes in mitochondria or plastids, or by the breaking down of the lipoprotein complex, when fat droplets separate out or the complex, in the fashion of oil droplets separating out of an emulsion.

The swelling of mitochondria and plastids can be demonstrated by observing affected cells in the living condition, in an isotonic solution. The breaking down of the lipoprotein complexes can only be shown by applying reagents that demonstrate either protein or fat.

After the tissues have been properly killed by reliable killing fluids and proper care has been taken not to mistake phenolic precipitates for fat bodies, fat or essential-oil inclusions may be detected by staining them gray with osmic-acid fixatives. Of course the fixative used must be one that does not tend to break down the lipoprotein complexes. The Meves killing fluid, made up of 2 per cent osmic-acid solution (5 parts per 100) and 1 per cent chromic-acid solution (15 parts per 100), may preserve cytoplasmic inclusions without altering them. The killing, however, is to be considered good only when mitochondria are well preserved, as they are much more fragile than plastids.

In normal citrus cells, from tissues that have been properly killed by the Meves fluid, mitochondria show as short rods and can be stained either by acid fuchsin or by haematoxylin. Plastids show as a well-defined, clear-cut network of lipoprotein material, easily stained bright red by acid fuchsin or solid black by haematoxylin. The lipoprotein network of the plastids enmeshes starch grains. A few tiny oil drops may separate out of the lipoprotein material itself.

Any gentle excitation of the cells in the living tissue may bring about a cytological response in the nature of an active multiplication or elongation of the mitochondria. This is shown for a citrus-canker lesion and for greasy spot or black melanose in Figures 1 and 2.

A more serious excitation may result in the breaking down of the lipoprotein complex in some plastids, or at least in parts of them. Part of a plastid may keep the staining reaction of the lipoprotein complex and stain sharply with acid fuchsin or haematoxylin, while the other part of the same plastid stains gray with osmic acid but does not stain with acid fuchsin or haematoxylin. This is shown in Figures 3 and 4 for a well-defined yellow spot of uncertain causation in grapefruit leaves. This fatty degeneration is generally concomitant with starch translocation from the affected plastid.

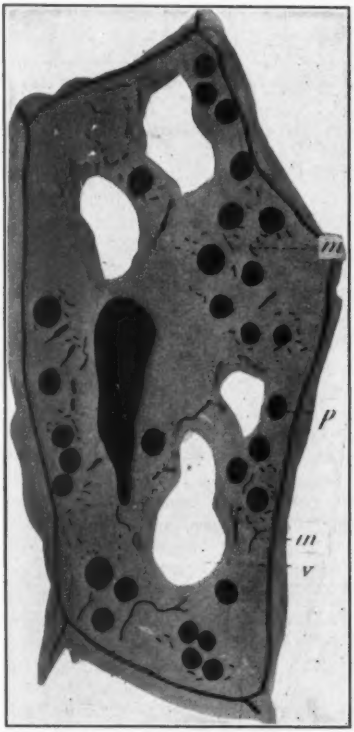


FIGURE 1.—Cell of an orange leaf, at the base of an experimental canker lesion resulting from the inoculation with *Pseudomonas citri* in the greenhouse at Gainesville, Fla. (Meves killing fluid, acid-fuchsin stain.) Mitochondria (*m*) show as long slender threads as contrasted with those of normal cells, which are in the form of short rods. Plastids (*p*) are very numerous and very small. Vacuoles (*v*) show as empty spaces

In short, most cytological phenomena in citrus tissues can be interpreted as the result of two physicochemical processes: (1) Exchanges of water from the cytoplasm (imbibition water) to vacuoles and (2) the breaking down of the lipoprotein complexes.

VACUOLES IN EPIDERMAL CELLS OF RIND OF CITRUS FRUITS

Whether citrus peel is green or yellowed, the epidermal cells are living and contain one big vacuole each. Any detrimental effect inducing injury to the fruit may cause the death of the epidermal cells. Even though the ordinary epidermal cells may have been killed and their vacuoles no longer stain with neutral red, the guard cells may continue to live around the stomata, and their vacuoles will readily stain with neutral red.

Certain stimuli such as Bordeaux-oil injury may result in the normally colorless vacuolar contents assuming a dark color (Fig. 5.) An excitation of the epidermal cell induced by such a stimulus as the light freezing resulting from exposure to a jet of liquid carbon dioxide causes the one big vacuole to break down into a number of small ones. (Fig. 6.) That vacuoles are liable to divide or to coalesce in the living cell was pointed out by Darwin for carnivorous plants. From that time it has been recognized to be of common occurrence in plant cells. Therefore any gentle stimulus, whatever the cause, may be expected to induce the normally single vacuole to break into many smaller ones. A more severe shock should result in more abrupt and irreversible changes in the vacuolar contents, as evidenced by its

browning in some cases. Death results in the breaking down of all cell constituents into a mass of droplets, as is shown for citrus cells injured by Bordeaux-oil spray.

CYTOLOGICAL CHANGES IN CELLS OF ORANGE PEEL COLORED BY TREATMENT WITH ETHYLENE GAS

Cells of Green Valencia Orange

Epidermal cells have one large vacuole, occupying most of the cell, and crowding the cytoplasm to a thin layer against the cell walls. The

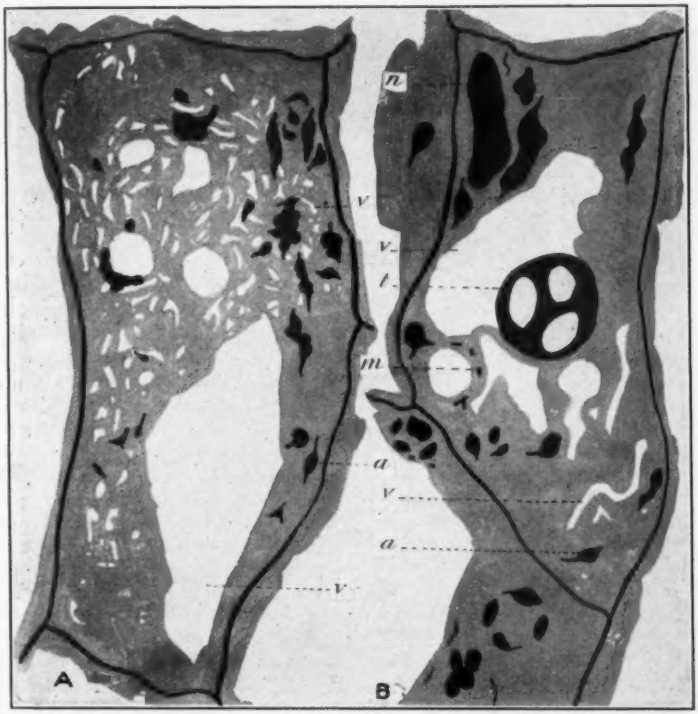


FIGURE 2.—Cells from greasy spots of grapefruit leaves (killed in Regaud fluid). In A, many mitochondria have differentiated into amyloplasts (*a*) and formed starch grains. Vacuoles (*v*) appear as numerous small areas in the grayish mass of the cytoplasm. When the killed cell shown as B was stained with haematoxylin and iodine plus potassium iodide the starch grains appeared amber yellow in the amyloplasts (*a*) and the large vacuolated tannic precipitate (*f*) bright yellow (stained by the potassium bichromate in the Regaud fluid). Mitochondria (*m*) show as black rods and vacuoles (*v*) as empty spaces; *n* is the nucleus

nucleus usually lies in the cytoplasmic layer at the bottom of the cell. A few mitochondria and plastids can be demonstrated in the cytoplasm.

Subepidermal cells and the cells of the next two layers are the ones that play active rôles in coloring the peel. In the green fruit these cells have one or several large vacuoles, but they are also rich in cytoplasm, with a number of chloroplasts. Each chloroplast is

heavily crowded with starch grains, even in the morning. The chloroplast, therefore, when examined in the living cell, shows as a network of green material embedding uncolored starch grains. In the green trabeculae between starch grains a few tiny oil drops are made evident by their high refractive power. Immersing a section of subepidermal tissues first in a solution of neutral red made isotonic by adding 10 per cent sugar, and second in a solution of indophenol blue, stains the vacuoles red and the oil drops blue.

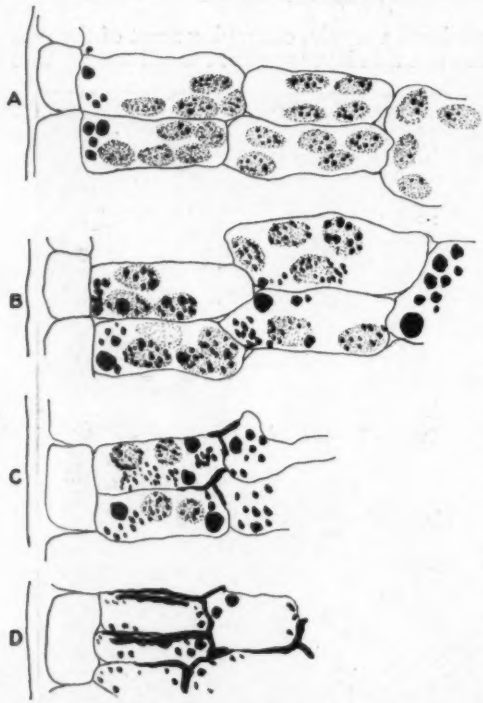


FIGURE 3.—Transverse sections of a grapefruit leaf, stained with indophenol blue, showing fatty degeneration of plastids in local areas, resulting in yellow spots. A.—Section at the margin of a lesion. Palisade cells and cells from spongy parenchyma show green chloroplasts, containing minute fatty inclusions. B.—Section from a more definitely affected portion. Chloroplasts in palisade cells show large fat inclusions. There are no chloroplasts in inner cells, where loose fat bodies are seen in the cytoplasm. C.—Section still farther in lesion. The fatty degeneration affects all but the subepidermal cells, where a few chloroplasts can still be demonstrated. D.—Section at center of lesion. Fatty degeneration affects all cells. The cell walls have absorbed fat substances and stain with indophenol blue. A large vacuole makes up most of the bulk of the cell, crowding the cytoplasm, with its fat inclusions, into a thin layer along the cell wall

CELLS OF ARTIFICIALLY COLORED VALENCIA ORANGE

Some Valencia oranges, picked when mature but still green in color, and similar to those that were studied for the cytology of the cells in the green fruit, were subjected for three days to the usual commercial ethylene-gas treatment for coloring fruit in the experimental plant of the United States Citrus-Disease Laboratory at Orlando, Fla., and then studied for the cytology of the cells of the peel. (Figs. 7 to 14.)

The epidermal cells show no very definite changes after the gassing process, beyond a few oil drops in the cytoplasm. The cells more than three or four layers deep in the peel do not seem to be affected to any extent. The most important changes occur in the subepidermal cells and the cells immediately below, where huge oil drops develop in the chloroplasts and the starch grains dwindle.

Many of the chloroplasts show a remarkable polarity, as they retain starch grains on one side and develop oil drops on the other. The yellow pigment, which is responsible for the development of the mature

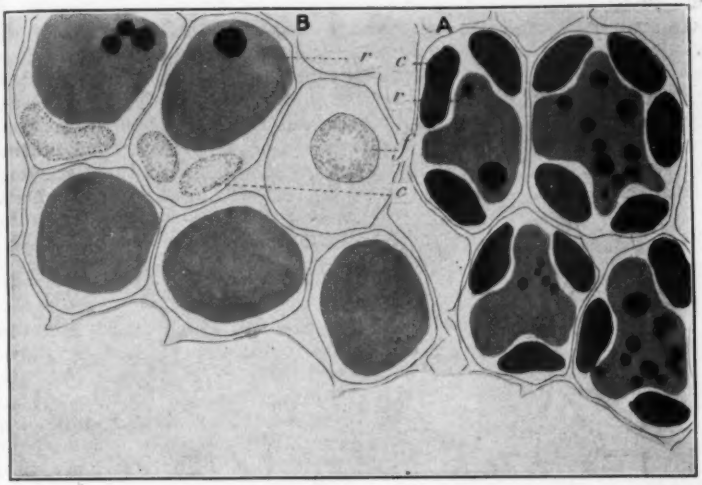


FIGURE 4.—A grapefruit leaf, stained vitally in neutral red in 10 per cent cane-sugar solution, to demonstrate vacuoles. A.—Cells of a green area showing chloroplasts (*c*) at the cell peripheries, the centers of the cells being occupied by the vacuoles (*r*). B.—Cells of a yellow spot in which the vacuole (*r*) occupies practically the entire volume of the cell. Some of the fat bodies (*f*) are present

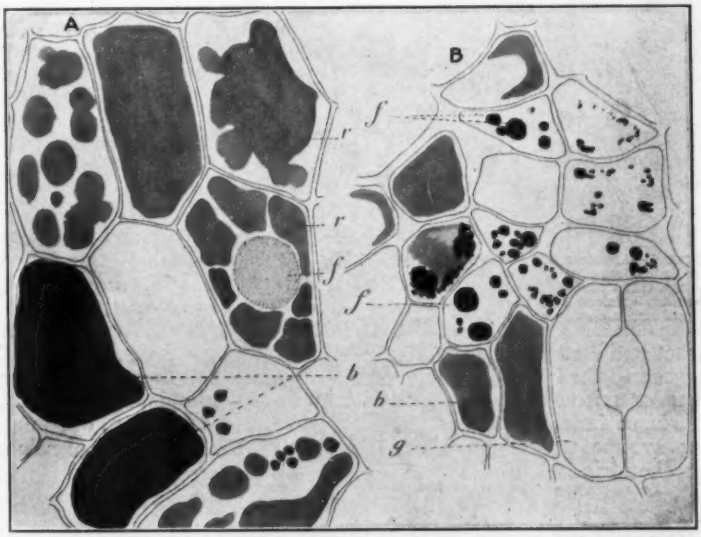


FIGURE 5.—Epidermis of orange fruits, showing injury from Bordeaux-oil spraying. A.—Cells from a discolored area. Some vacuoles (*b*) show dark-colored contents, which are responsible for the stained appearance of the fruits. Neighboring cells contain uncolored vacuoles (*r*). These vacuoles can be readily demonstrated by vital staining with neutral red. Red-staining vacuoles are numerous and small in each epidermal cell around the brown area, whereas the normal epidermal cell contains only one large vacuole; *f* is a fat globule. B.—Portion of the epidermis from a dark-stained area stained by indophenol blue. It demonstrates fatty inclusions (*f*) as blue bodies. These are represented black in the figure and stand out sharply from the shaded areas that represent the pigmented vacuolar content (*b*) of some of the cells; *g* is a guard cell of a stoma

fruit color, is in a solution in the oil drops. These cytological processes are not morbid processes and do not result in the death of the cells, although some chloroplasts may seem to break down and set free a number of oil drops in sections of subepidermal tissues of yellowed fruits, but this aspect may result from the cells in the colored fruits being more fragile and collapsing easily as the sections are being handled. That subepidermal cells of fully colored mature fruits remain living is demonstrated by the fact that their vacuoles stain easily by neutral red. It really seems that the gassing treatment has a stimulating effect on mitochondria in the cells.

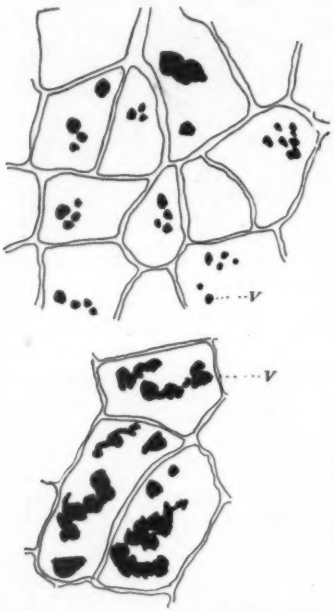


FIGURE 6.—Appearance of the vacuolar system (v) in epidermal cells of a mature Valencia orange as the result of gentle freezing experimentally obtained by exposing it to a jet of liquid carbon dioxide for 10 seconds

EFFECT OF SELENIUM SALTS ON ORANGE PEEL

A most striking development of a deep red-orange pigment has been obtained by H. R. Fulton, by treating oranges with solutions of sodium selenite or selenium dioxide, in concentrations of about 1 per cent.

Fulton kindly made the specimens available for cytological study. In sharp contrast with the development of the normal orange color, which results from an orange fat-soluble pigment accumulating within oil drops in the chloroplasts, the selenium-induced deeply colored spotting results from mitochondria secreting an orange-red pigment.

Subepidermal cells from such selenium-stained areas, when examined in the living condition in an isotonic solution, were observed to be crowded with minute orange grains. Treating similar cells by mitochondrial methods, killing them by the Meves fluid, and staining them by acid fuchsin demonstrate that mitochondria in the cells have the same aspect and distribution as the orange-colored grains in the living cell, suggesting that these orange grains are mitochondria secreting pigment, or mitochondria differentiating into chromoplasts. (Fig. 15.) This is, so far as the writer knows, a unique phenomenon, opening a new avenue to the experimental study of the physiological rôle of mitochondria.

FRENCHING

Frenching being made evident by the discoloration of certain areas of leaves, cytological studies might have been expected to demonstrate alterations of chloroplasts and mitochondria. In fact, as contrasted with cells from green areas of frenched leaves, where chloroplasts may be demonstrated in killed tissues as networks of deeply staining

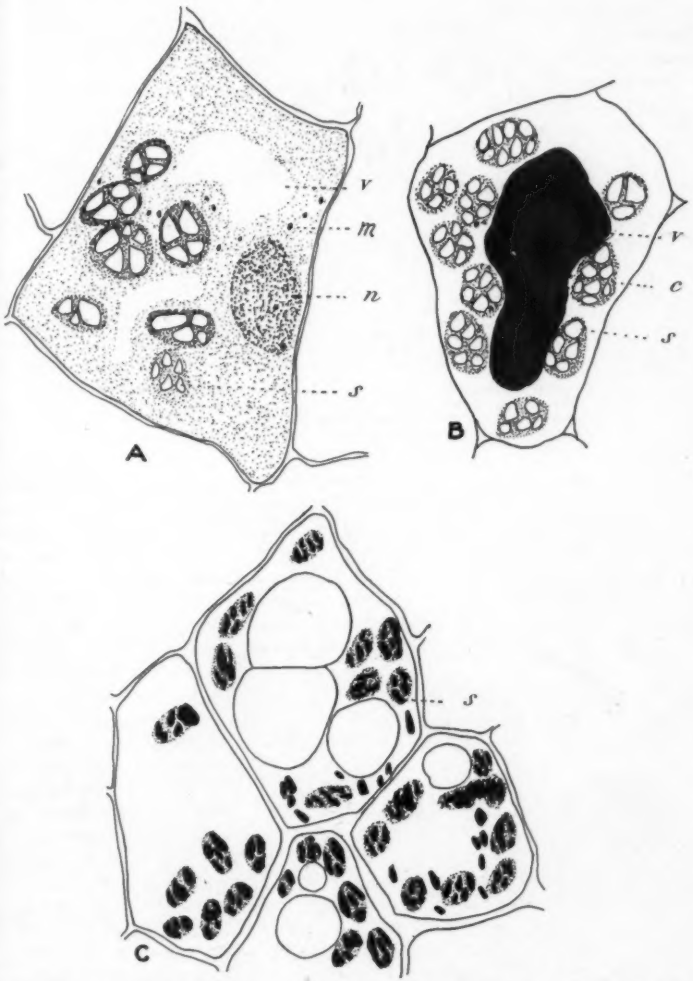


FIGURE 7.—Normal subepidermal cells of green oranges. A.—From section of tissue killed by Meves fluid and stained with acid fuchsin. B.—Picked from tree at 11 a. m. and stained vitally by neutral red in 9.5 per cent solution of cane sugar. C.—Same as B stained in iodine plus potassium-iodide solution. Starch grains appear as dark bodies in the chloroplasts. Throughout this figure *n* indicates nucleus; *c*, chloroplast; *m*, mitochondrium; *v*, vacuole; and *s*, starch grain

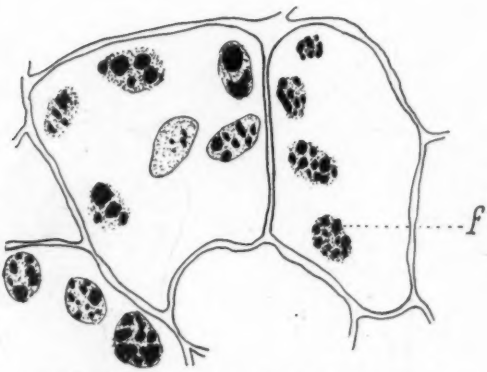


FIGURE 8.—Subepidermal cells of a Valencia orange that had developed an orange color after treatment with ethylene gas for three days. Sections of peel stained by indophenol blue have fat bodies (*f*) as blue globules in the plastids

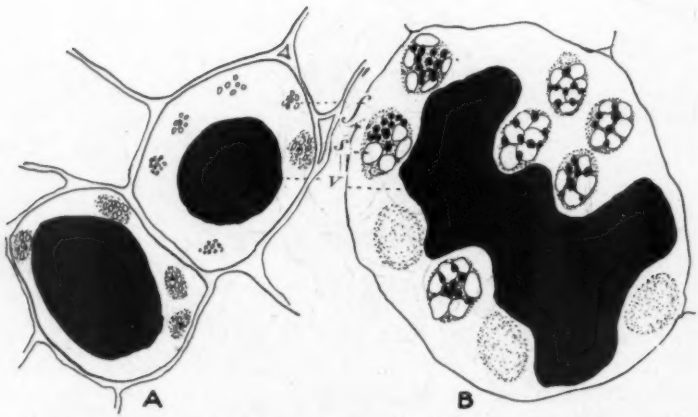


FIGURE 9.—A gassed orange. A.—Section of peel. The vacuoles (*v*) have been stained vitally by neutral red, demonstrating that the cells of the peel remain in perfect condition in the gassed fruit, but chloroplasts show symptoms of fatty degeneration; *f*, fat bodies. B.—Subepidermal cells. The vacuole (*v*) stained red vitally by neutral red and in the chloroplasts the fat globules (*f*) subsequently stained blue by indophenol blue. The starch grains remained unstained. This aspect, obtained by vital plus postvital stainings, corresponds to that of the same tissues killed by Meves fluid

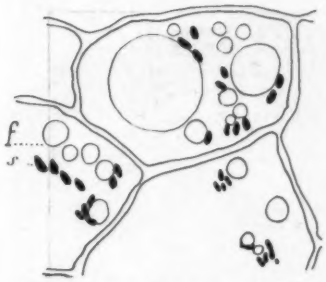


FIGURE 10.—Ultimate stages of fatty degeneration of chloroplasts in some cells of the peel of gassed fruits; *s*, starch grains stained by iodine plus potassium iodide; *f*, fat globules

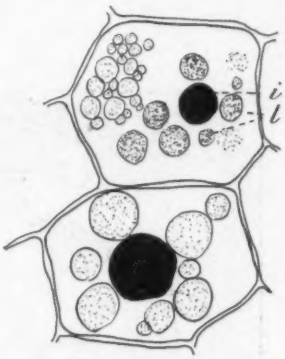


FIGURE 11.—Same kind of tissues as shown in Figure 10, demonstrating some fat and essential-oil inclusions. Some of these inclusions (*i*) stained blue with indophenol blue and others (*l*) stained a light lavender blue

FIGURE 12.—Subepidermal cells from Valencia oranges (killed by Meves fluid and stained by acid fuchsin). A.—Normal green. Mitochondria, (*m*) figured black, showed as deep-red grains in the cytoplasm; plastids showed as a red network embedding starch grains (*s*). Tiny fat globules (*f*) show in the plastids. B.—Gassed. Many plastids contain starch grains (*s*) toward one pole, while the other pole undergoes fatty degeneration (*f*). These plastids show as a black network embedding amber-colored starch grains on one side and dark-colored fat globules on the other. Mitochondria (*m*) show as dark grains or rods in the cytoplasm. Some mitochondria divide and others form amyloplasts. It looks as though gas treatment acted as a stimulus to mitochondrial activity. The vacuoles (*v*) are stuffed with phenolic compounds

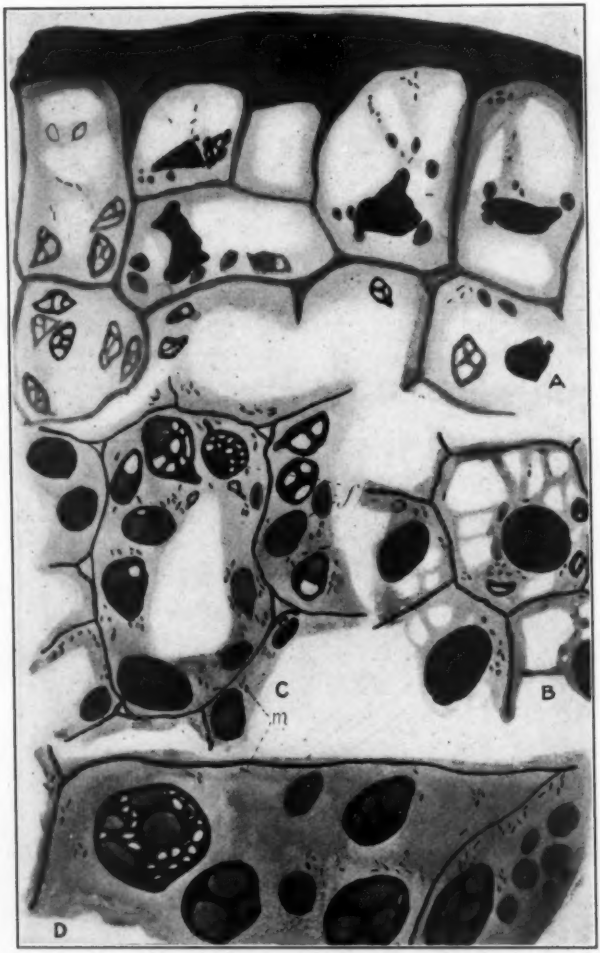


FIGURE 13.—A.—Section of epidermal and subepidermal cells of a gassed Valencia orange. B to D.—Tangential sections: B, Epidermal cells; C and D, subepidermal cells. When killed by Meves fluid and stained by acid fuchsin, the mitochondria (*m*), which look black in the figure, showed as red grains or rods. Plastids have a reticulated appearance and are colored gray. A few small fatty inclusions (*f*) show as grayish grains in the cytoplasm itself

lipoprotein complex, embedding starch grains, plastids from light-colored or white areas show all forms of alterations or may even have failed to develop. (Figs. 16–18.)

Mitochondria, instead of showing the normal rodlike appearance, may assume a swollen, vesiculated appearance, and show as hyaline, hollow spheres. Where mitochondria show such changes, no plastids can be expected to form in the cell, as plastids are only the ultimate form of differentiating mitochondria, and swollen mitochondria are conspicuously unable to develop.

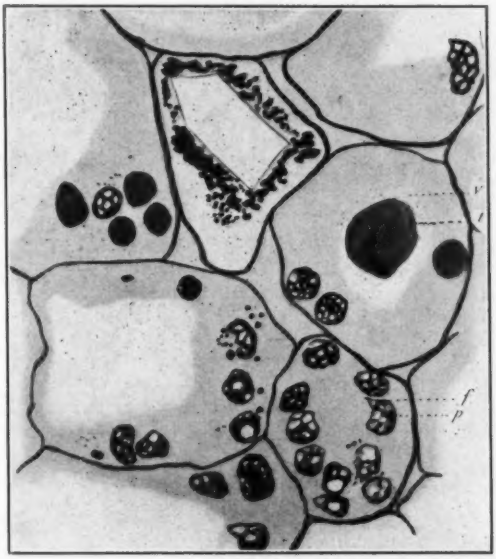


FIGURE 14.—Tangential section through rows of subepidermal cells of peel of gassed Valencia orange. Tissues killed by Meves fluid and stained by iron haematoxylin and iodine plus potassium iodide. The plastids (*p*) have a reticulated appearance and contain no starch grains. Various stages of fatty degeneration (*f*) are represented. Some cells contain tannoid precipitates (*t*) within vacuoles (*v*)

Where plastids form in leaves affected by frenching they generally show a swollen diffuse appearance, without any clear-cut contour. They do not stain well with either acid fuchsin or haematoxylin, but stain light gray with osmic acid. These swollen plastids may contain a few small starch grains, which often appear to have been partially digested. (Fig. 17.)

The abnormalities in leaves affected by frenching are not limited to the palisade cells, and the cells near to the under surface of the leaf may develop tyloses. (Fig. 17.)

EFFECT OF BORON

Specimens of citrus leaves developed symptoms of mottling as the result of being fed boron, during T. Ralph Robinson's experiments in the greenhouse of the Bureau of Plant Industry. Mr. Robinson

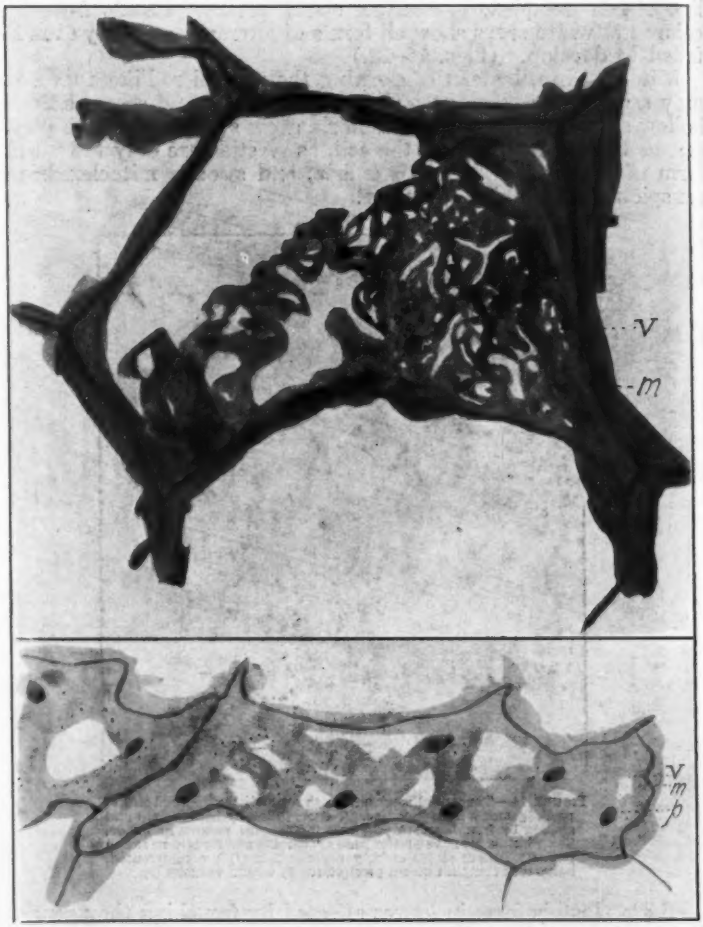
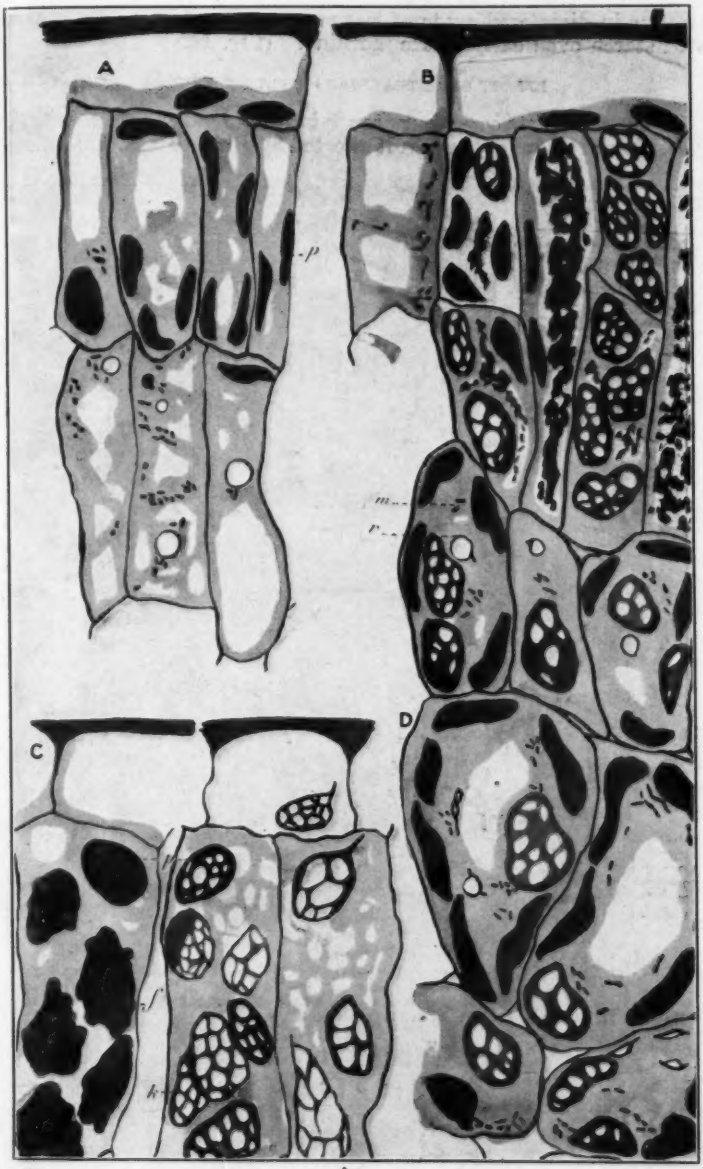


FIGURE 15.—Subepidermal cells of an orange, from a lesion developed as the result of a treatment with selenium dioxide solution. Mitochondria (*m*), which show in the living condition as bright-red rods, can be preserved by killing the cell with the Meves fluid, and can be stained with acid fuchsin. Vacuoles (*v*) show as empty places within the cytoplasm. Plastids (*p*), when they occur, are restricted in size

EXPLANATORY LEGEND FOR FIGURE 16

FIGURE 16.—Frenching of orange leaves. A and B.—Killed by Meves fluid and stained by iron haematoxylin. Transverse sections through epidermal and palisade cells. The cells of A show a few plastids (*p*) or none and only unstained spheres and a number of mitochondria. In the cells of B the plastids show as a network of fatty material. Some mitochondria (*m*) show the normal appearance of rods, but others are swollen and vesiculated and look like ring bodies (*r*). Vacuoles (*v*) are full of tannic precipitates. C and D.—Two stages of fatty degeneration of subepidermal cells, killed by Meves fluid and stained by acid fuchsin. Cells of C from green tissue. The plastids (*p*) show as solid protein bodies with some gray-colored fatty inclusions (*f*). Cells of D from frenching. One plastid still colored as a solid network (*p*), but most of them stained gray with osmic acid (*k*), demonstrating fat reaction. Only a small portion of some plastids can still be stained red (represented black in the figure)



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kindly submitted material to the writer for cytological study, and plastids in discolored areas of leaves were observed to be crowded with starch even early in the morning. (Fig. 19.)

EFFECT OF TETRANICHUS CITRI PUNCTURES

Specimens of leaves of sour orange infested by *Tetranichus citri* and specimens of leaves of grapefruit affected by purple scale were collected

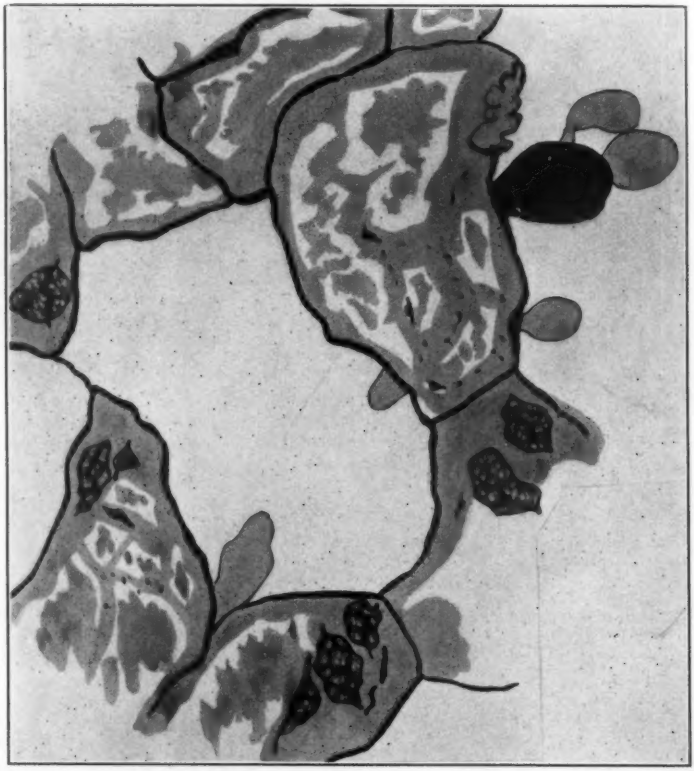

FIGURE 17.—Frenching of grapefruit. Leaf tissue killed by Meves fluid and stained by haematoxylin and iodine plus potassium iodide. Section of cells from spongy parenchyma at the under surface of the leaf. Cells send tyloses into lacunae between them. Plastids show as black network embedding starch grains. Starch is represented gray

in the grove of the United States Citrus-Disease Field Laboratroy at Orlando. The material was identified by W. W. Yothers and killed for cytological work.

The results, as illustrated in the case of *Tetranichus citri*, show that changes are induced around the cells which have been actually punctured. These changes are similar to those observed in the case of frenching. (Fig. 20.)

Other abnormalities have been studied in citrus; for instance, various cases of variegation have been submitted. Their effects are similar to those of mottling which have been described in this paper. (Fig. 21.)

SUMMARY

Cells of green parts of citrus leaves or fruits normally contain one large vacuole, which can be stained in the living cell by using neutral red in 10 per cent cane-sugar solution as a dye. Gentle excitations tend to cause the large vacuole to break into a number of smaller vacuoles. Greater shock may result in the browning of the vacuolar content and ultimately in the collapse of the cell, when the vacuolar material is thrown out of colloidal states and mixed with the cytoplasmic constituents on which it exercises a coagulating effect.

Cells of green parts of citrus contain in their cytoplasm short rod-like mitochondria and starch-forming chloroplasts.

A number of conditions may result in the breaking down of the lipoprotein complex of which the normal mitochondria and plastids are made, resulting in such cytological phenomena as are naturally observed in the leaf tissues affected by frenching, or such as may be experimentally induced in the peel of the fruit by the ethylene-gas treatment for artificial coloring.

FIGURE 18.—Frenching of grapefruit leaf tissues killed by Meves killing fluid and stained by iron haematoxylin and iodine plus potassium iodide: A.—Tangential section. B.—Transverse section. Some plastids show the normal appearance of a black network embedding amber-colored starch grains (*s*), but others become a swollen, vesiculated mass of fatty material. The vacuolar content has been precipitated by the killing fluid into a number of small dark grains. These vacuolar precipitates (*pe*) roughly outline the contour of the vacuoles

The natural coloring process of the fruit is concomitant with starch translocation from the chloroplasts in the cells of the three upper layers in the peel. As starch disappears, fat bodies develop in the chloroplasts, and the orange pigment that gives the fruit its color

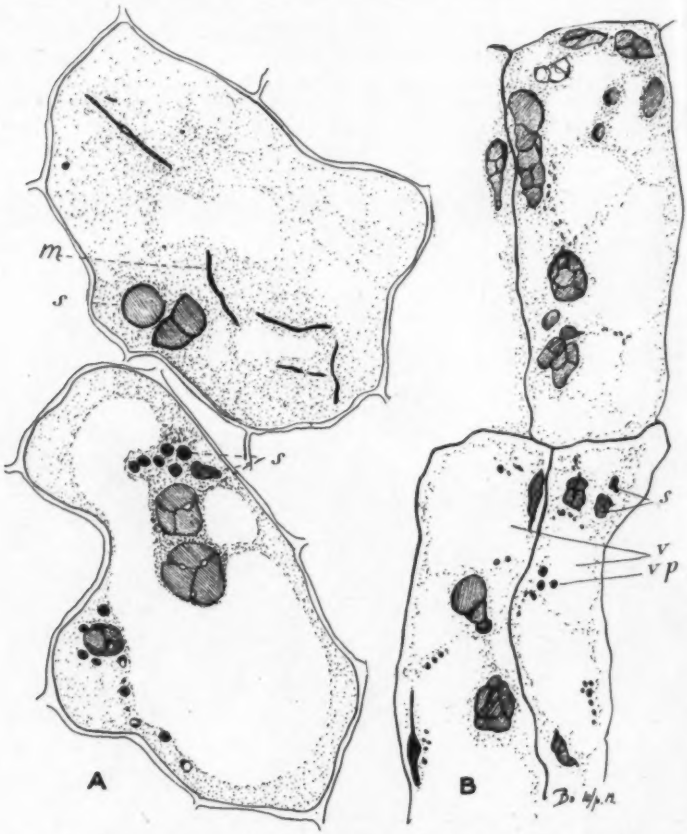

FIGURE 19.—Rough lemon leaf showing effects of boron. A.—Cells of spongy parenchyma discolored by watering with 5 parts per million solution of boron. In one of the cells mitochondria (*m*) are conspicuous, but in the other cell all mitochondria have differentiated into amyloplasts which are stuffed with starch grains (*s*). B.—Palisade cells discolored by watering with a 10 parts per million solution of boron (Robinson's experiments). Plastids are heavily crowded with starch grains (*s*) even in the early morning, as if translocation of starch was inhibited by the effect of boron. Some vacuolar precipitates (*vp*) show in the vacuoles (*v*)

goes into solution in the fat bodies inside the chloroplasts. The same result can be obtained artificially through gas treatment.

The action of selenium salts may induce mitochondria that seem to be normally inactive, to develop red pigment.

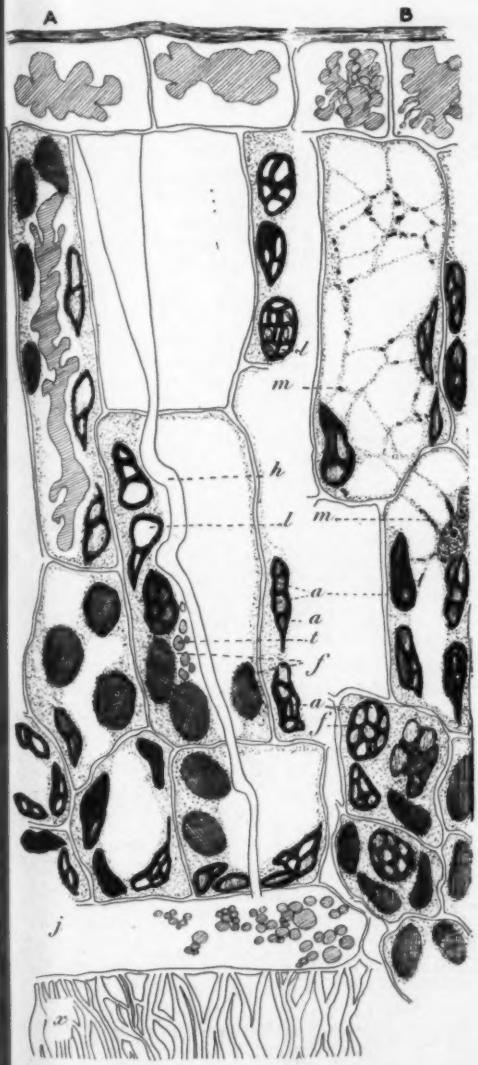

FIGURE 20.—Transverse sections (A and B) of a sour-orange leaf, showing effect of *Tetranichus citri* puncture. The trace of the puncture (*h*) is seen from the epidermis down to the phloem (*j*). Affected cells and neighboring cells show a fatty (*f*) degeneration of some plastids leaving empty spaces (*l*). Some plastids, however, still show starch grains (*a*). Affected cells show phenolic precipitates in the vacuoles (*t*). Section B shows cells a few layers away from the puncture; plastids are densely crowded with starch grains, mitochondria (*m*) stain sharply in the cytoplasm, and no phenolic compounds are seen in the vacuoles; *z* is part of the xylem

FIGURE 21.—Transverse section of dilute-green area of variegated leaf of Bittersweet orange. Palisade cells and cells of the inferior layers of spongy parenchyma contain no green plastids. They contain only fat globules (*f*). Internal cells show green plastids (*p*); most of the plastids are crowded with fat globules (*f*). ox, Of the numerous crystals of calcium oxylate; *e* is one of epidermal cells

ANTINEURITIC AND WATER-SOLUBLE B VITAMINS IN BEEF AND PORK[1]

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INTRODUCTION

Recent experiments by Smith and Hendrick (*9*),[2] by Goldberger and his associates (*3*), and by others (*1*, *6*, *7*, *8*, *10*) seem to have demonstrated that the growth-promoting water-soluble B vitamin consists of at least two factors, namely, (1) the antineuritic vitamin, labile to heat, and (2), a heat-stabile vitamin. Neither vitamin in the absence of the other will promote growth in rats even though the diet is adequate in other respects. In previous papers (*4*, *5*) the writer, as a result of feeding experiments with pigeons, reported that lean pork was an excellent source of vitamin B (antineuritic vitamin), but that beef contained a much smaller proportion of this vitamin. In view of the present knowledge concerning the multiple nature of water-soluble B it is evident that the antineuritic value of lean pork or beef is not necessarily an indication of the amount of the vitamin B complex present. For this reason a study has been made of the relative amounts of the antineuritic and the composite water-soluble B vitamins in lean pork and beef by feeding tests with pigeons and with rats, respectively. The results of these experiments are reported in this paper.

Earlier publications concerning the antineuritic and water-soluble B vitamins have been reviewed by the writer (*4*, *5*). After the second paper had been submitted for publication, Wright (*11*) reported the results of experiments concerning the effects of cold storage on vitamin B in meats. Fresh and cold-storage beef, pork, lamb, and mutton were tested in feeding experiments with young chicks. These experiments did not disclose any material difference with respect to growth-promoting vitamin B among the different kinds of meat.

ANTINEURITIC VITAMIN

METHODS

The amounts of the antineuritic vitamin in samples of beef and pork were compared by feeding tests with vigorous adult homing pigeons. Six birds, as a rule, were used to test each ration. Each pigeon was forcibly fed daily, except Sunday, an amount equivalent to 5 per cent of the weight of the bird at the beginning of the test. The pigeons were weighed before the test began and at weekly intervals thereafter, always before feeding.

In each series of experiments, as a control, at least one pen of pigeons was fed a basal ration practically free from the antineuritic

[1] Received for publication Dec. 17, 1928; issued April, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 446.

vitamin, but otherwise adequate for maintenance. The ration was made up as follows:

Basal ration for pigeons

	Per cent
Polished rice	79. 7
Beef residue	7. 0
Hydrogenated cottonseed oil	7. 3
Ash mixture	4. 0
Cod-liver oil	2. 0
	100. 0

The beef residue was obtained in the preparation of bacterial culture media. Ground lean beef was mixed with water and heated three hours at 60° C. The mixture was strained through muslin and the residue was pressed as dry as practicable. This beef residue was then heated 1.5 hours in an autoclave at 15 pounds steam pressure to destroy any antineuritic vitamin present. The material was finally dried in a current of warm air and finely ground.

The hydrogenated cottonseed oil was a well-known commercial product. The cod-liver oil was of medicinal grade. The ash mixture was made up according to a formula cited by Drummond and Watson (*2*). This ration contained approximately 10 per cent fat and 12 per cent protein.

Rations including samples of beef or pork to be tested for the antineuritic vitamin were made up according to the same formula except that the proportions of beef residue, cottonseed oil, and starch were adjusted so that each ration contained 10 per cent fat and at least 12 per cent protein.

In order to compare the antineuritic values of beef and pork with some product generally recognized as rich in the antineuritic vitamin, control groups of pigeons were fed rations containing different proportions of dried bakers' and of dried brewers' yeast, respectively.

DESCRIPTION OF SAMPLES

Five lots of high-grade round steak were purchased from three local packing houses and given laboratory numbers 1687, 1698, 1705, 1707, and 1712, respectively. Sample No. 1687 was used in the first experiment with pigeons and samples Nos. 1705 and 1707 were used in the second experiment. Samples Nos. 1687, 1698, 1705, and 1712 were tested in experiments with rats.

Three lots of fresh and two lots of smoked hams were purchased from two local packing houses. The fresh hams were given laboratory numbers 1684, 1697, and 1708 and the smoked hams numbers 1704 and 1713, respectively. Sample No. 1684 was used in the first experiment and samples Nos. 1704 and 1713 were used in the second experiment with pigeons. All the samples were tested in experiments with rats.

Dried bakers' yeast (No. 1693) was purchased from a prominent manufacturer of fresh and dried yeast. The dried brewers' yeast (No. 1702) was obtained from a firm which supplies various products for use in vitamin investigations. The two lots of yeast were tested both with pigeons and with rats.

PREPARATION OF MEAT FOR FEEDING TESTS

The lean meat, both beef and pork, was trimmed as free from fat as practicable, ground, mixed with toluol and water, and spread out in thin layers in shallow galvanized-iron pans. The pans were then placed in a drying oven and the meat was dried in a rapidly moving current of warm air, the temperature not exceeding 60° C. The dried meat was ground, glaced in covered containers, and stored in a cold room at 32°–36° F. Each lot of dried meat was analyzed for nitrogen and fat before it was mixed in a ration.

EXPERIMENT 1: ANTINEURITIC VITAMIN IN BEEF AND PORK

The results of experiment 1 are presented in Table 1. The pigeons that were fed the basal ration practically free from antineuritic vitamin (pen 1) behaved in a characteristic manner. Five birds developed the usual symptoms of polyneuritis while the sixth became extremely thin and died without showing symptoms of the disease.

TABLE 1.—*Results of feeding pigeons various quantities of fresh ham and beef as their sole source of the antineuritic vitamin*

Ration *	Pigeon No.	Duration of test period	Weight of pigeon at start	Change in weight during test	Result
		Days	*Grams*	*Per cent*	
Pen 1—Basal ration (12 per cent protein)	37	19	281	−1.7	Polyneuritis.
	38	63	271	−48.0	Died.
	39	23	282		Polyneuritis.
	40	28	347	−15.3	Do.
	41	41	320	−24.7	Do.
	42	25	341	−13.8	Do.
Average		33	307	−17.3	
Pen 2—5 per cent dried fresh ham No. 1684 (12 per cent protein).	43	70	281	−14.7	Thin but active.
	44	70	276	+5.8	Fine condition.
	45	70	415	+14.0	Do.
	46	70	330	−18.2	Thin but active.
	48	70	360	+13.6	Fine condition.
Average		70	332	+.1	
Pen 3—10 per cent dried fresh ham No. 1684 (15 per cent protein).	49	44	262	+9.5	Died.
	51	70	341	+8.5	Fine condition.
	52	70	336	+8.6	Do.
	53	70	284	+10.6	Good condition.
	54	70	400	+2.3	Fine condition.
	55	70	341	+20.8	Do.
Average		66	327	+10.1	
Pen 4—20 per cent dried beef No. 1687 (22.6 per cent protein).	56	66	276	−21.7	Polyneuritis.
	57	35	390	−9.5	Do.
	58	42	380	−12.6	Do.
	59	28	296	−35.1	Do.
	60	49	319	−13.5	Do.
	61	66	384	−10.9	Do.
Average		48	341	−17.2	
Pen 5—40 per cent dried beef No. 1687 (38.8 per cent protein).	62	70	308	−.7	Fine condition.
	64	70	356	−2.5	Good condition.
	65	70	284	+4.3	Do.
	66	70	348	+.6	Fine condition.
	67	70	313	−2.6	Good condition.
	68	34	296	−26.3	Died.
Average		64	318	−4.5	

* The percentage of dried beef or pork in a ration is expressed in terms of fat-free, dried meat.

In pen 2 the ration containing 5 per cent of dried fresh ham protected five pigeons against polyneuritis for at least 70 days. At the end of the test the average weight of the birds was practically the same as at the beginning.

Ten per cent of dried fresh ham likewise protected five pigeons in pen 3 against polyneuritis for at least 70 days. One bird died on the forty-fourth day without showing symptoms of the disease. Postmortem examination disclosed no pathological changes, but the intestinal tract was heavily infested with a parasite common to pigeons.

Even 20 per cent of dried beef, however, failed to protect the pigeons in pen 4 against polyneuritis and loss in weight. All the birds developed the characteristic symptoms of the disease and lost considerable weight. Forty per cent of dried beef, however, protected five of the six pigeons in pen 5 for at least 70 days against both polyneuritis and material loss in weight. One bird died on the thirty-fourth day without apparent symptoms of disease. No postmortem examination was made, as the bird had been dead some time when found.

EXPERIMENT 2: ANTINEURITIC VITAMIN IN BEEF, PORK, AND YEAST

In Table 2 are reported the results of the second experiment with pigeons. In this experiment the ground polished rice was heated 1.5 hours at 15 pounds steam pressure to destroy any antineuritic vitamin present, whereas in the first experiment the rice was not heated. In the second experiment the pigeons fed the basal ration (pen 6) developed polyneuritis considerably earlier than in the previous test, but the loss in weight was greater in the first experiment. Apparently the polished rice contained a small amount of antineuritic vitamin which, in experiment 2, was destroyed on heating.

The dried bakers' yeast tested (No. 1693) did not prove to be a good source of the antineuritic vitamin. Five per cent did not prevent polyneuritis and even 7.5 per cent was not much more effective. (See Table 2, pens 7 and 8.)

Dried brewers' yeast (No. 1702) was much more effective than the bakers' yeast against polyneuritis and loss in weight. Five per cent of the brewers' yeast protected five of the six pigeons in pen 9 against polyneuritis for at least eight weeks, but one bird developed the disease on the fortieth day. On an average the pigeons fed this ration practically maintained their weight.

The dried fresh beef tested in this experiment appeared to contain only a moderate amount of the antineuritic vitamin, as will be seen from the data for pens 10, 11, and 12, Table 2. Even 34.2 per cent, the maximum proportion fed, was not quite sufficient to protect pigeons for eight weeks against polyneuritis. One pigeon receiving the maximum percentage of beef developed polyneuritis and another died, while four survived. On an average the birds in this pen maintained their weight.

Smoked ham, like the fresh ham in experiment 1, proved to be an excellent source of the antineuritic vitamin, as will be seen in the data for pens 13, 14, and 15 in Table 2. Even 5 per cent of the dried smoked ham protected all the pigeons in pen 13 against polyneuritis for at least eight weeks, while the average gain in weight for that pen was 6.8 per cent.

TABLE 2.—*Results of feeding various quantities of beef, pork, and yeast to pigeons as their sole source of the antineuritic vitamin*

Ration [a]	Pigeon No.	Duration of test period	Weight of pigeon at start	Change in weight during test	Result
		Days	*Grams*	*Per cent*	
Pen 6—Basal ration (12 per cent protein)	1	38	394	−32.7	Died.
	2	19	376	−8.8	Polyneuritis.
	3	28	417	−15.6	Do.
	4	14	359	−2.8	Do.
	5	22	418	−7.9	Do.
	6	16	338	−3.6	Do.
Average		23	384	−11.9	
Pen 7—5 per cent dried bakers' yeast No. 1693 (12.4 per cent protein).	7	22	354	+1.7	Polyneuritis.
	8	47	381	−24.9	Do.
	9	33	368	−6.5	Do.
	10	23	447	−8.1	Do.
	11	56	395	−17.5	Very thin.
	12	36	367	−2.7	Polyneuritis.
Average		36	385	−9.7	
Pen 8—7.5 per cent dried bakers' yeast No. 1693 (12.2 per cent protein).	13	56	349	+12.9	Fair condition.
	14	41	355	−4.5	Polyneuritis.
	15	37	439	−11.6	Died.
	16	47	366	−22.7	Do.
	17	36	361	+7.8	Polyneuritis.
	18	38	425	−10.1	Died.
Average		43	383	−4.7	
Pen 9—5 per cent dried brewers' yeast No. 1702 (12.4 per cent protein).	19	56	341	−13.5	Thin.
	20	56	414	−4.3	Fine condition.
	21	56	401	+7.0	Do.
	22	40	384	+4.7	Polyneuritis.
	23	56	369	−3.0	Good condition.
	24	56	418	+6.4	Fine condition.
Average		53	388	−.5	
Pen 10—12.8 per cent dried beef No. 1705 (16.4 per cent protein).	25	29	358	−5.6	Died.
	26	56	370	−23.2	Very thin.
	27	17	392	−3.1	Polyneuritis.
	28	22	379	−2.9	Do.
	29	33	347	−20.2	Do.
	30	28	424	−2.6	Do.
Average		31	378	−9.6	
Pen 11—21.4 per cent dried beef No. 1705 (23 per cent protein).	31	31	386	+.5	Polyneuritis.
	35	34	439	−11.6	Do.
	61	40	345	−11.6	Do.
	62	55	418	−17.5	Good condition.
	63	42	352	−10.2	Polyneuritis.
	64	55	413	−15.3	Good condition.
Average		43	392	−10.9	
Pen 12—34.2 per cent dried beef No. 1705 (33.4 per cent protein).	37	28	403	−1.5	Died.
	38	45	404	+.5	Polyneuritis.
	39	56	392	−6.9	Good condition.
	40	56	399	+2.8	Fine condition.
	41	56	376	+13.3	Do.
	42	56	357	−7.6	Fair condition.
Average		50	389	+.1	
Pen 13—5 per cent dried smoked ham No. 1704 (11.9 per cent protein).	43	56	420	+.7	Fine condition.
	44	56	350	−1.1	Do.
	45	56	398	+9.8	Do.
	46	56	378	+17.5	Do.
	47	56	331	+9.1	Do.
	48	56	438	+5.0	
Average		56	386	+6.8	

[a] The percentage of dried beef or pork in the ration is expressed in terms of fat-free dried meat.

TABLE 2.—*Results of feeding various quantities of beef, pork, and yeast to pigeons as their sole source of the antineuritic vitamin*—Continued

Ration	Pigeon No.	Duration of test period	Weight of pigeon at start	Change in weight during test	Result
		Days	*Grams*	*Per cent*	
Pen 14—10.5 per cent dried smoked ham No. 1704 (13 per cent protein).	49	56	437	+16.7	Fine condition.
	50	56	359	+19.2	Do.
	51	56	416	+16.6	Do.
	52	45	395	+8.9	Died.
	53	56	348	+15.8	Fine condition.
	54	56	350	+17.4	Do.
Average		54	384	+15.8	
Pen 15—17.5 per cent dried smoked ham No. 1704 (17.4 per cent protein).	55	49	358	+12.6	Broken leg; removed.[b]
	56	56	360	+16.1	Fine condition.
	57	56	394	+15.7	Do.
	58	56	417	+25.9	Do.
	59	56	388	+17.7	Do.
	60	56	362	+4.7	Do.
Average		56	384	+16.0	

[b] Data for this pigeon not included in average.

WATER-SOLUBLE B VITAMINS[a]

METHODS

The so-called water-soluble B vitamin was estimated by feeding tests with young albino rats. The rats were fed a basal ration practically free from water-soluble B vitamin until they ceased to gain in weight. They were then fed a ration containing a definite proportion of the product to be tested. The rats, which had been raised in this laboratory, were selected from litters containing not more than eight nor fewer than six animals. Only rats weighing at least 40 gm. each when 28 days old were used. The rats fed a single ration were selected from at least two and usually from three litters. Each rat was kept in an individual cage with a raised screen bottom. The ration was fed in a self-feeder and an accurate record was kept of the amount consumed.

Basal ration for rats

	Per cent
Casein (N×6.25)	20.0
Ash mixture	4.0
Cod-liver oil	2.0
Hydrogenated cottonseed oil	8.0
Cassava starch to make	100.0

The casein, a commercial product, was purified by repeated extraction with 60 per cent by volume alcohol. The cassava starch was not purified, as it had been found to be practically free from water-soluble B. The ash mixture, cod-liver oil, and cottonseed oil were the same products as those used in the tests with pigeons. This ration was adequate for growth except for lack of water-soluble B.

Rations containing samples of meat to be tested for water-soluble B were made up according to the same formula except that the proportions of casein, cottonseed oil, and starch were adjusted so that each ration contained 10 per cent fat and at least 20 per cent protein.

[a] The writer thanks George G. Snider, of the Biochemic Division, for assistance in the feeding experiments with rats.

As in the experiment with pigeons, the percentage of meat in the ration is expressed in terms of the fat-free dried product.

EXPERIMENT 3: WATER-SOLUBLE B VITAMINS IN PORK

Three lots of fresh hams and two lots of smoked hams were tested for water-soluble B vitamins by feeding experiments with rats. The growth curves of the rats fed the fresh ham are shown in Figures 1 and 2 and those of the rats fed smoked ham in Figures 3 and 4. Certain experimental data are presented in Table 3.

TABLE 3.—*Record of feed consumed and weight gains made by rats fed various quantities of fresh and smoked ham as their source of water-soluble B vitamins*

Ration	Rat No.	Sex	Duration of test period	Total gain in weight	Average daily gain in weight	Total feed eaten	Total pork eaten	Average amount of pork eaten daily	Average gain in weight per gram of feed
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
20 per cent dried fresh ham No. 1684.	938 A	Male	53	205	3.9	653	130.6	2.46	0.31
	938 B	Female	48	109	2.3	443	88.6	1.85	.25
	938 C	do	50	93	1.9	447	89.4	1.79	.21
	938 D	Male	50	179	3.6	572	114.4	2.29	.31
	938 E	do	49	131	2.7	451	90.2	1.84	.29
30 per cent dried fresh ham No. 1684.	942 A	do	46	153	3.3	543	162.9	3.54	.28
	942 B	Female	44	117	2.7	434	130.2	2.96	.27
	942 C	do	44	122	2.8	431	129.3	2.94	.28
	942 D	do	36	102	2.8	409	122.7	3.41	.25
10 per cent dried fresh ham Nos. 1697 and 1708.	944 A	Male	59	129	2.2	480	48.0	.81	.27
	944 B	Female	60	104	1.7	508	50.8	.85	.20
	944 C	Male	59	152	2.6	577	57.7	.98	.26
	944 D	Female	63	109	1.7	503	50.3	.80	.22
	944 F	Male	60	119	2.0	438	43.8	.73	.27
15 per cent dried fresh ham Nos. 1697, 1708.	954 A	do	60	242	4.0	674	101.1	1.69	.36
	954 C	do	60	180	3.0	611	91.7	1.53	.29
	954 D	do	60	169	2.8	618	92.7	1.55	.27
	954 E	Female	60	96	1.6	531	79.7	1.33	.18
	954 F	do	60	115	1.9	544	81.6	1.36	.21
10.5 per cent dried smoked ham No. 1704.	949 A	Male	60	99	1.7	464	48.7	.81	.21
	949 B	do	60	104	1.7	468	49.1	.82	.22
	949 C	Female	60	90	1.5	447	46.9	.78	.20
	949 D	Male	60	119	2.0	540	56.7	.95	.22
	949 F	do	60	153	2.6	596	62.6	1.04	.26
17.5 per cent dried smoked ham No. 1704.	950 A	do	60	131	2.2	557	97.5	1.63	.24
	950 C	do	60	133	2.2	602	105.4	1.76	.22
	950 D	do	60	178	3.0	773	135.3	2.26	.23
	950 E	Female	60	119	2.0	651	113.9	1.90	.18
24 per cent dried smoked ham No. 1704.	955 A	Male	46	188	4.1	569	136.6	2.97	.33
	955 B	do	46	154	3.3	510	122.4	2.66	.30
	955 C	do	46	173	3.8	511	122.6	2.67	.34
	955 D	do	44	158	3.6	563	135.1	3.07	.28
	955 F	Female	45	104	2.3	366	87.8	1.95	.28
10 per cent dried oked ham No. 1713.	967 A	Male	62	132	2.1	585	58.5	.94	.23
	967 B	do	62	145	2.3	585	58.5	.94	.25
	967 D	Female	62	96	1.5	482	48.2	.78	.20
	967 E	do	62	118	1.9	534	53.4	.86	.22
15 per cent dried smoked ham No. 1713.	969 A	Male	60	184	3.1	657	98.6	1.64	.28
	969 B	do	60	156	2.6	624	93.6	1.56	.25
	969 C	do	60	194	3.2	656	98.4	1.64	.30
	969 D	do	60	194	3.2	727	109.1	1.82	.27

The percentage of dried pork in the rations ranged from 10 to 30. Referring to Figures 2, 3, and 4, one observes that 10 per cent of dried fresh ham (fig. 2) or a like percentage of dried smoked ham (figs. 3 and 4) furnished sufficient water-soluble B vitamins for moderate but not for normal growth.

Fifteen per cent of dried fresh ham (fig. 2) and a like percentage of dried smoked ham (fig. 4) each supplied an adequate amount of

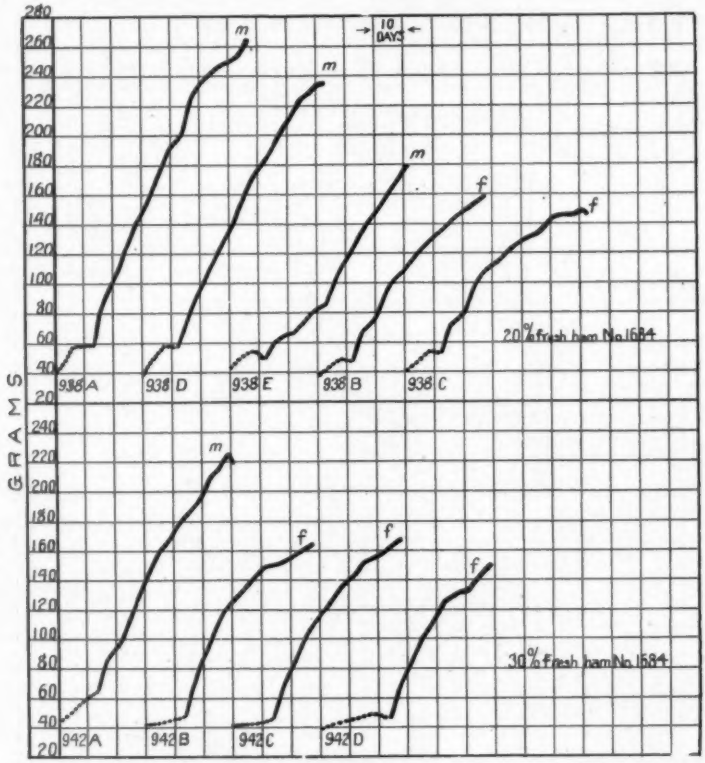

FIGURE 1.—Growth curves of two groups of rats fed rations containing 20 and 30 per cent, respectively, of dried fresh ham as the source of water-soluble B vitamins. The dotted lines indicate growth on the basal ration; the heavy continuous lines growth when the rations contained pork. The percentage of pork is expressed in terms of the fat-free dried meat. The letters *m* and *f* denote sex

water-soluble B vitamins for excellent growth in rats. Seventeen and one-half per cent of dried smoked ham induced excellent growth in one male and one female rat and moderate growth in two others (fig. 3). Larger proportions of fresh ham (fig. 1) and of smoked ham (fig. 3) naturally supplied adequate amounts of water-soluble B vitamins.

EXPERIMENT 4: WATER-SOLUBLE B VITAMINS IN BEEF

Four lots of dried fresh beef were tested for water-soluble B vitamins. The growth curves of the rats fed these samples of beef are shown in Figures 5 and 6 and certain related data are presented in

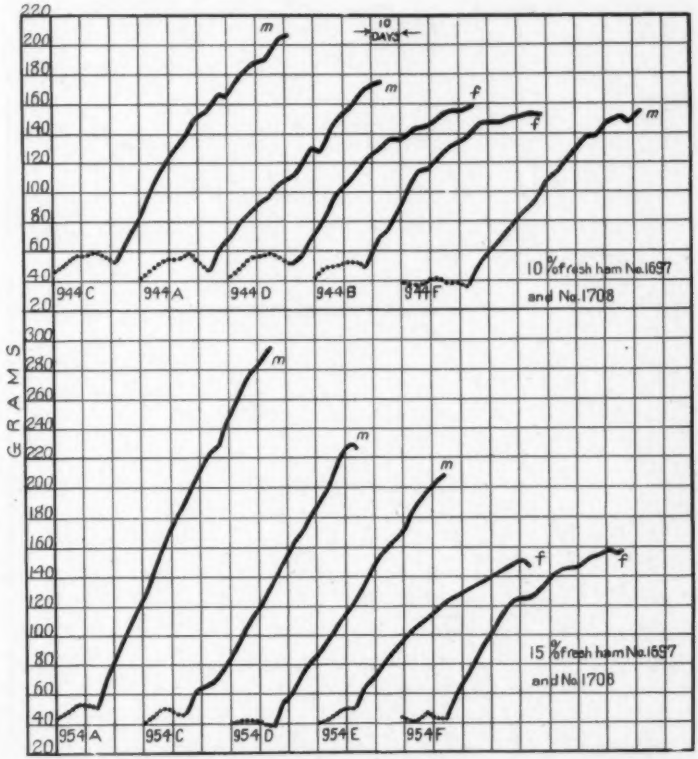

FIGURE 2.—Growth curves of two groups of rats fed rations containing 10 and 15 per cent, respectively, of dried fresh ham as the source of water-soluble B vitamins. The dotted lines indicate growth on the basal ration; the heavy continuous lines growth when the rations contained pork. The percentage of pork is expressed in terms of the fat-free dried meat

Table 4. From Figure 5 it is apparent that 40 per cent of dried beef No. 1687 did not furnish sufficient water-soluble B vitamins for material growth, but when the beef was increased to 70 per cent the rats grew rapidly.

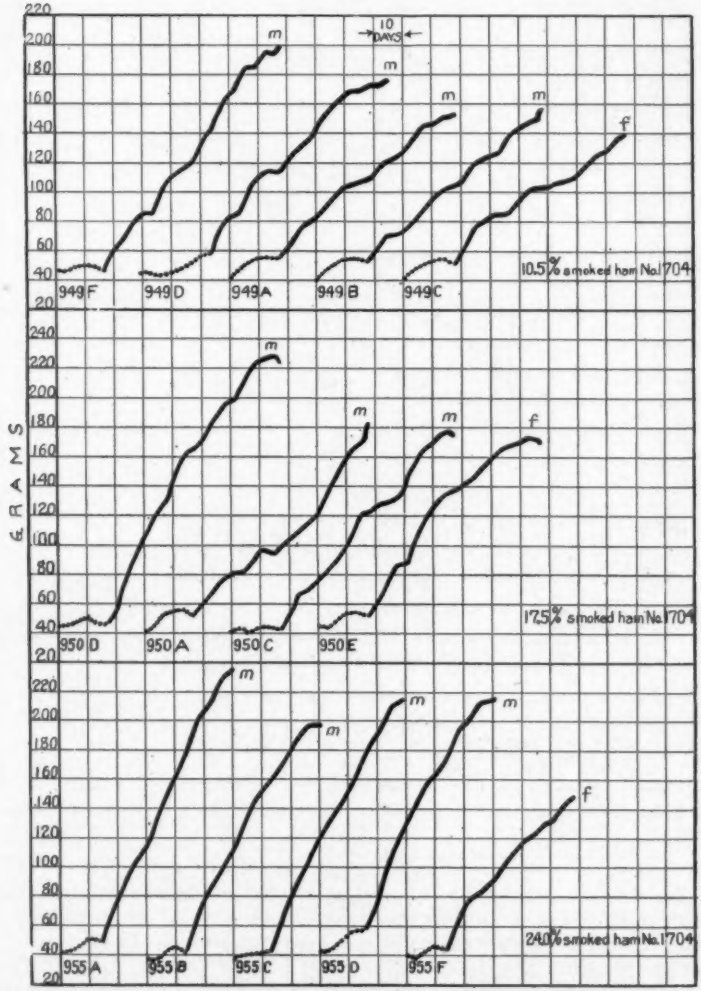

FIGURE 3.—Growth curves of three groups of rats fed rations containing 10.5, 17.5, and 24 per cent, respectively, of dried smoked ham as the source of water-soluble B vitamins. The dotted lines indicate growth on the basal ration; the heavy continuous lines growth when the rations contained pork. The percentage of pork is expressed in terms of the fat-free dried meat

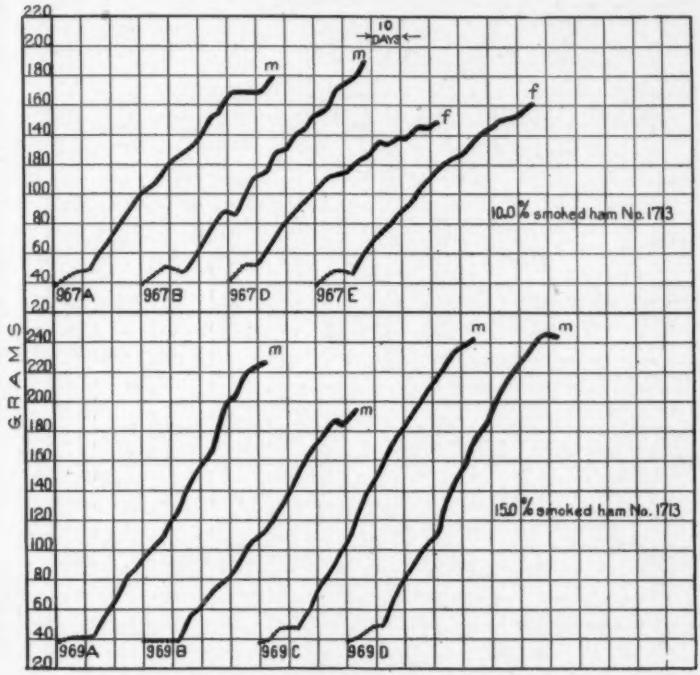

FIGURE 4.—Growth curves of two groups of rats fed rations containing 10 and 15 per cent, respectively, of dried smoked ham as the source of water-soluble B vitamins. The dotted lines indicate growth on the basal ration; the heavy continuous lines growth when the rations contained pork. The percentage of pork is expressed in terms of the fat-free dried meat

Twenty-six per cent of another lot of dried beef (No. 1705) did not promote satisfactory growth in 4 out of 5 rats (fig. 5), but 39 per cent of beef No. 1705 induced excellent growth in 3 out of 4 rats.

Twenty per cent of the third lot of beef (No. 1698) stimulated only slight growth in rats, but when the beef was increased to 40 per cent, four of the six rats made excellent growth. Two females failed to respond.

EXPERIMENT 5: WATER-SOLUBLE B VITAMINS IN YEAST

For purposes of comparison, one lot each of dried bakers' and of dried brewers' yeast were tested for water-soluble B vitamins. The growth curves of the rats fed these samples are shown in Figure 7 and certain related data are presented in Table 5. The brewers' yeast in the proportion of 5 per cent and the bakers' yeast in the proportion of 7.5 per cent furnished sufficient water-soluble B vitamins for excellent growth.

TABLE 4.—*Record of feed consumed and weight gains made by rats fed various quantities of beef as a source of water-soluble B vitamins*

Ration	Rat No.	Sex	Duration of test period	Total gain in weight [a]	Average daily gain in weight	Total feed eaten	Total beef eaten	Average amount of beef eaten daily	Average gain in weight per gram of feed
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
40 per cent dried beef No. 1687.	928 A	Male	21	−2		88	35.2	1.68	
	928 C	do	21	24	1.1	128	51.2	2.44	0.19
	928 D	do	21	12	.6	105	42.0	2.00	.11
	928 E	do	21	11	.5	98	39.2	1.87	.11
70 per cent dried beef No. 1687.	928 A	do	38	180	4.7	442	309.4	8.14	.41
	928 C	do	42	129	3.1	407	284.9	6.78	.32
	928 D	do	38	133	3.5	368	257.6	6.78	.36
	928 E	do	39	149	3.8	411	287.7	7.38	.36
20 per cent dried beef No. 1698.	945 A	do	24	31	1.3	112	22.4	.93	.28
	945 B	Female	28	23	.8	114	22.8	.81	.20
	945 C	Male	21	26	1.2	111	22.2	1.06	.23
	945 D	do	24	44	1.8	155	31.0	1.29	.28
40 per cent dried beef Nos. 1698 and 1712.	946 A	do	63	219	3.5	723	289.2	4.59	.30
	946 B	Female	11	−8		36	14.4	1.31	
	946 C	Male	60	159	2.7	522	208.8	3.48	.30
	946 D	Female	25	13	.5	87	34.8	1.39	.15
	946 E	do	60	126	2.1	605	242.0	4.03	.21
	946 F	do	59	130	2.2	534	213.6	3.62	.24
26 per cent dried beef No. 1705.	959 A	Male	50	152	3.0	553	143.8	2.88	.27
	959 B	do	24	−3		97	25.2	1.05	
	959 C	Female	53	84	1.6	419	108.9	2.05	.20
	959 D	do	42	79	1.9	373	97.0	2.31	.21
	959 E	do	38	7	.2	161	41.9	1.10	.04
39 per cent dried beef No. 1705.	960 A	Male	60	272	4.5	839	327.2	5.45	.32
	960 B	do	63	192	3.0	741	289.0	4.59	.26
	960 D	Female	63	89	1.4	506	197.3	3.13	.18
	960 E	do	63	124	2.0	656	255.8	4.06	.19

[a] Minus sign denotes loss.

TABLE 5.—*Record of feed consumed and weight gains made by rats fed various quantities of yeast as a source of water-soluble B vitamins*

Ration	Rat No.	Sex	Duration of test period	Total gain in weight [a]	Average daily gain in weight	Total feed eaten	Total yeast eaten	Average amount of yeast eaten daily	Average gain in weight per gram of feed
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
3 per cent dried bakers' yeast No. 1693.	936 A	Male	30	21	0.7	155	4.7	0.16	0.14
	936 B	Female	30	6	.2	106	3.2	.11	.06
	93[illegible] C	Male	30	−14		107	3.2	.11	
	936 D	do	27	−7		84	2.5	.09	
	936 E	do	30	−6		111	3.3	.11	
5 per cent dried bakers' yeast No. 1693.	937 A	do	52	74	1.4	386	19.3	.37	.19
	937 C	do	52	66	1.3	392	19.6	.38	.17
	937 D	do	30	7	.2	121	6.1	.20	.06
	937 E	do	42	31	.7	215	10.8	.26	.14
	937 F	do	39	86	2.2	278	13.9	.36	.31
7.5 per cent dried bakers' yeast No. 1693.	948 A	do	63	214	3.4	803	60.2	.96	.27
	948 B	do	63	147	2.3	606	45.5	.72	.24
	948 D	do	63	200	3.2	770	57.8	.92	.26
	948 E	do	63	162	2.6	675	50.6	.80	.24
3 per cent dried brewers' yeast No. 1702.	948 A	do	14	−3		48	1.4	.10	
	948 B	do	14	−9		38	1.1	.08	
	948 D	do	21	21	1.0	109	3.3	.16	.19
	948 E	do	14	−5		48	1.4	.10	
5 per cent dried brewers' yeast No. 1702.	935 A	do	60	214	3.6	663	33.2	.55	.32
	935 B	Female	57	107	1.9	425	21.3	.37	.25
	935 C	Male	60	216	3.6	600	30.0	.50	.36

[a] Minus sign denotes loss.

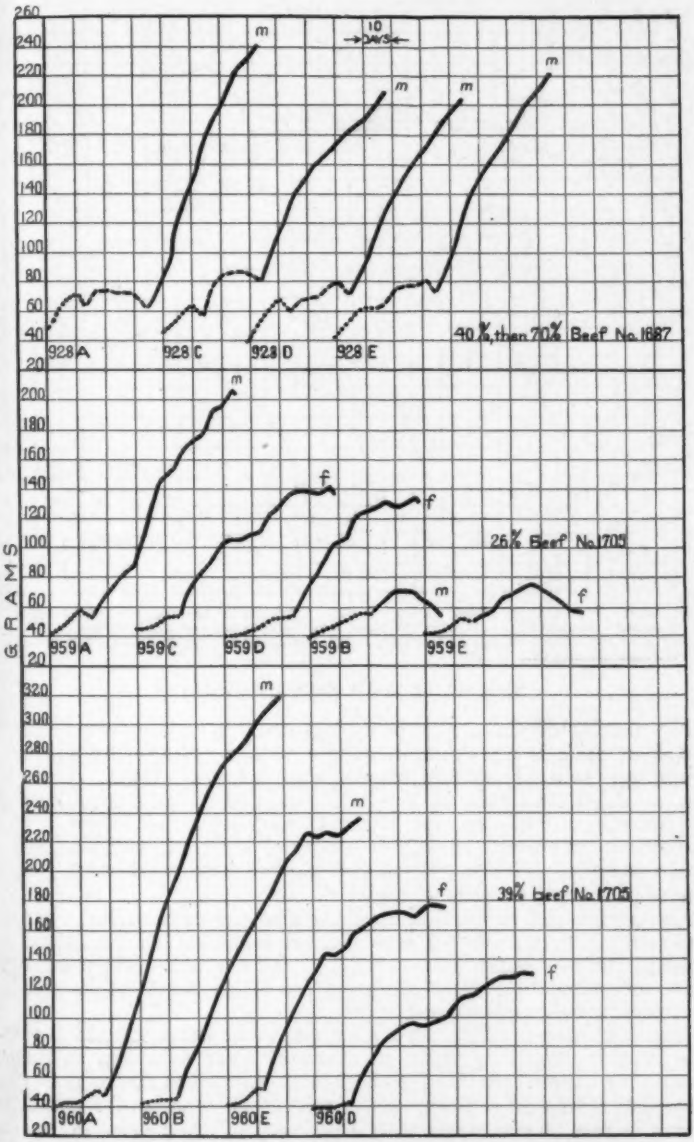

FIGURE 5.—Growth curves of three groups of rats fed dried fresh beef as the source of water-soluble B vitamins. With the first group of rats numbered 928 the dotted lines indicate growth on the basal ration, the broken lines growth on the ration containing 40 per cent of dried beef, and the heavy continuous lines growth when the ration contained 70 per cent of dried beef. With the two other groups of rats the dotted lines indicate growth on the basal ration, and the heavy continuous lines growth on the rations containing dried beef. The percentage of beef is expressed in terms of the dried fat-free meat

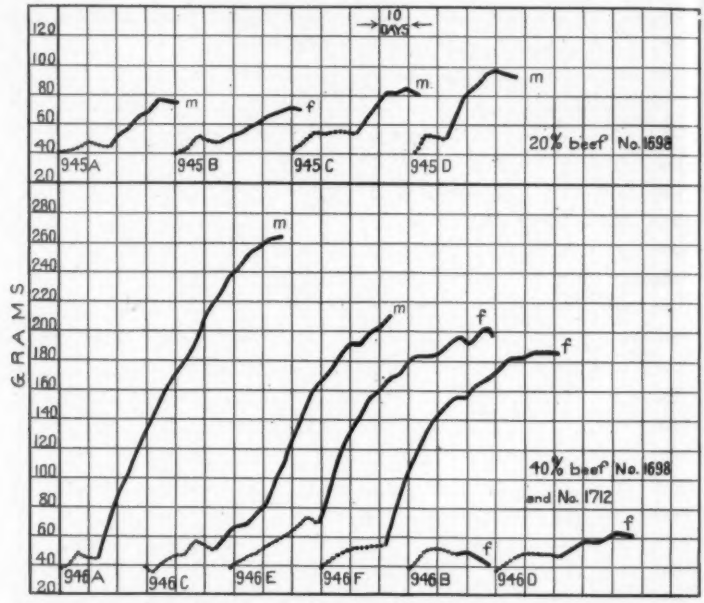

FIGURE 6.—Growth curves of two groups of rats fed dried beef as the source of water-soluble B vitamins. The percentage of beef in the rations is expressed in terms of dried fat-free meat. The dotted lines indicate growth on the basal ration, the heavy continuous lines growth on the rations containing beef

SUMMARY AND CONCLUSIONS

The results of the experiments reported in this paper indicate that lean pork is an excellent source of the antineuritic vitamin, comparing favorably in this respect with brewers' yeast. Five per cent of dried lean pork in a ration protected pigeons against both polyneuritis and loss in weight for eight weeks and longer. This proportion corresponds to a daily intake of 1 gm. of dried pork for a pigeon weighing 400 gm. Fresh and smoked ham were equally good sources of the antineuritic vitamin.

Beef was found to contain much less of the antineuritic vitamin than pork, from 35 to 40 per cent of dried, lean beef being required to protect pigeons against polyneuritis for eight weeks. These proportions correspond to a daily intake of from 7 to 8 gm. of dried beef for a pigeon weighing 400 gm.

Lean pork was found to be a good source of water-soluble B vitamins, but it did not contain so much of these vitamins as either brewers' or bakers' yeast. From 15 to 25 per cent of dried lean pork in the diet furnished sufficient water-soluble B vitamins for excellent growth in rats as compared with 5 per cent of dried brewers' yeast. In terms of the average daily intake during a period of 60 days approximately 1.6 gm. of dried lean pork or 0.5 gm. of dried brewers' yeast proved to be adequate for excellent growth in male rats. No material difference was observed between fresh and smoked hams as

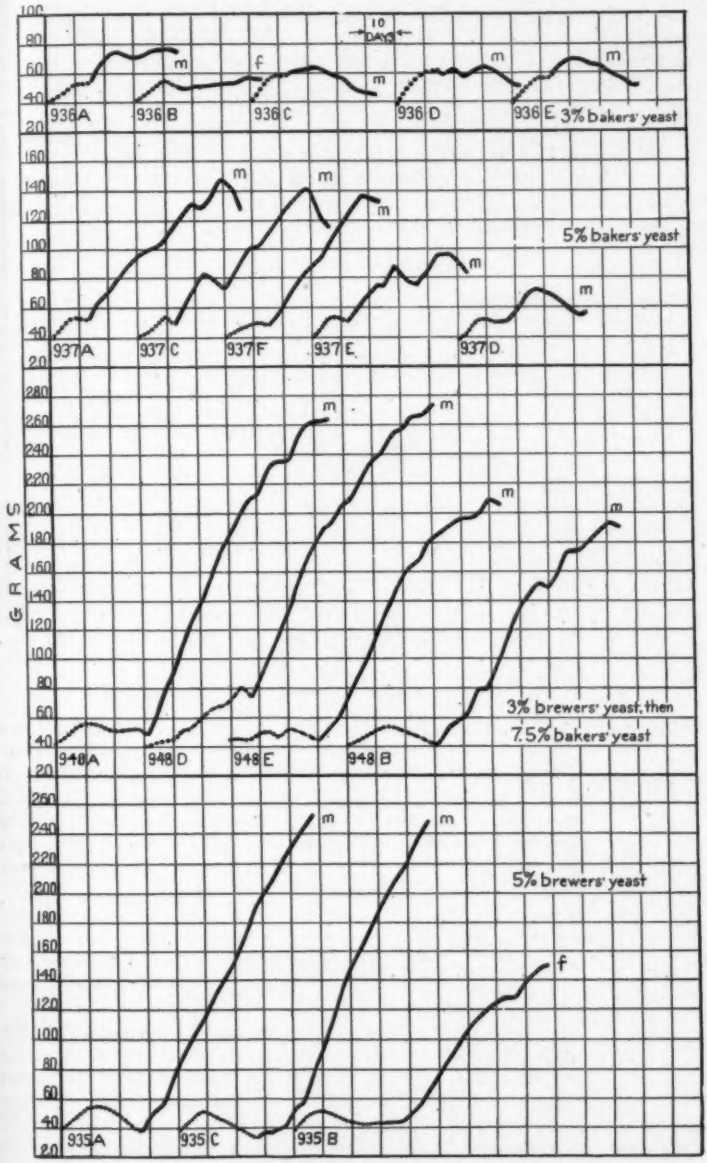

FIGURE 7.—Growth curves of four groups of rats fed rations containing different percentages of dried bakers' and brewers' yeast as sources of water-soluble B vitamins. The dotted lines indicate growth on the basal ration, the broken lines (rats 948) growth on a ration containing 3 per cent of brewers' yeast, and the heavy continuous lines growth when the rations contained bakers' or brewers' yeast

sources of the water-soluble B vitamins. The fact that dried pork was approximately as rich in the antineuritic vitamin as dried brewers' yeast, while the latter was from 3 to 4 times richer in water-soluble B vitamins than the former, indicates that the dried brewers' yeast was correspondingly richer in the heat-stabile vitamin than dried, lean pork.

Lean beef contained much less water-soluble B vitamins than lean pork. From 40 to 70 per cent of dried fresh beef was required for excellent growth in rats as compared with 15 to 25 per cent of dried lean pork or 5 per cent of dried brewers' yeast.

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THE TRANSLOCATION OF POTASSIUM IN TOMATO PLANTS AND ITS RELATION TO THEIR CARBOHYDRATE AND NITROGEN DISTRIBUTION [1]

By GEORGE JANSSEN, *Assistant Agronomist*, and R. P. BARTHOLOMEW, *Assistant Agronomist, Arkansas Agricultural Experiment Station*

INTRODUCTION

During the past decade much attention has been given by plant physiologists to the study of the rôle of the various plant-food elements in plant nutrition. The proper balancing of the nutrient solution for plant species and for the same plant at various stages of its growth has to a great extent depended upon a knowledge of the functions and actions of the various elements in plant growth. The progress in agriculture in recent years in the study of the practical fertilization of soils has also in a large measure rested upon the knowledge of the actions of the elements in plant growth. This knowledge has further contributed toward the proper balancing of plant food in the soil solution.

From the standpoint of agricultural production, the value of a specific interpretation of the action of the nitrogen and carbohydrate balance in the promotion of vegetativeness and fruitfulness, as found by Kraus and Kraybill (*15*),[2] can not be overemphasized. The further development of this study carried on by Murneek (*21*) on correlations in the tomato plant is very stimulating. MacGillivray (*19*) has shown the relationship between nitrogen, carbohydrate, and the phosphorus content of the plant. He found that in the case of the tomato plant phosphorus may be redistributed in a phosphorus-starved plant, that the highest concentration of phosphorus was located in the regions of growth, and that the size of the plant was directly dependent upon the phosphorus available to it. These and like results tend also to show that not one but several factors in a culture solution may function to bring about similar types of growth.

For the past few years the writers have been working on the minimum concentrations of potassium necessary for the growth of various crops. In such a study the action of potassium in the plant is important. It was thought, therefore, that the work might profitably be extended to include translocation studies of the potassium ion in the plant as well as its relation to carbohydrate and protein synthesis. The objects of the experiment here reported were to study (1) the lability of the potassium ion; that is, how readily it could be translocated in the plant; (2) the effect of a decreased amount of potassium in plants growing in a potash-deficient nutrient solution; and (3) the correlations which might be obtained between certain of the carbohydrate compounds and potassium as well as between the nitrogen and potassium content of the plant.

[1] Received for publication Nov. 28, 1928; issued April, 1929. Contribution from the department of agronomy, Arkansas Agricultural Experiment Station; published with the permission of the director.

[2] Reference is made by number (italic) to "Literature cited," p. 464.

METHODS

Norton wilt-resistant tomato plants were used in this work. The plants were grown in 2-gallon glazed jars. These jars were filled with a high grade of sand which by chemical analyses was found to contain no soluble and only traces of insoluble potassium salts. The sand was then watered with a modified form of Knop's nutrient solution used by Kraus and Kraybill (*15*), MacGillivray (*19*), Murneek (*21*), and others. The stock solutions used in the first series contained the following compounds:

Solution 1:
- 2 per cent $CaSO_4$.
- 5 per cent $Ca(NO_3)_2.4H_2O$.
- 2 per cent $(NH_4)_2SO_4$.

Solution 2:
- 2 per cent $MgSO_4.7H_2O$.
- 2 per cent $CaH_4(PO_4)_2.H_2O$.
- 0.2 per cent $MnSO_4$.

Solution 3: 3 per cent KCl.

For the second series of the experiment the nutrient solution was altered so as to decrease the amount of $Ca(NO_3)_2$, the nitrogen being replaced with $(NH_4)_2SO_4$ and the calcium with $CaCl_2$. This was done largely to maintain a greater hydrogen-ion concentration, thereby keeping other salts in solution. The following solutions were used in the second series of experiments:

Solution A:
- 2 per cent NH_4NO_3.
- 3 per cent $(NH_4)_2SO_4$.
- 2 per cent $CaSO_4$.
- 3 per cent $CaCl_2$.

Solution B:
- 2 per cent $CaH_4(PO_4)_2.H_2O$.
- 2 per cent $MgSO_4.7H_2O$.
- 0.1 per cent $MnSO_4$.
- 0.05 per cent boric acid.

Solution C: 3 per cent KCl

The nutrient solutions were diluted 1 to 6 before they were applied to the plants. Thus 1 liter of A was diluted with 5 liters of distilled water; and 1 liter of B was diluted with 5 liters of water where no potassium was applied and 4 liters of water together with 1 liter of C where the potassium was a constituent of the solution, making in the end a 1 to 6 solution. In each series 5 c. c. of a 0.1 per cent solution of ferric tartrate was added to each 1,000 c. c. of solution. In a good many instances the solutions applied were a little stronger than the above formulae provide, but since the jars were watered daily from the top there was a continual dilution of the nutrient solution within the jars.

The tomato seeds were sown in 2-gallon jars containing sand, before any nutrient solution was added. After emergence, the plants were thinned down to approximately 16 to the jar, and when they had reached a height of 1 to 2 inches the nutrient solution plus potassium was added to the jars. When the plants had developed to a height of 6 inches they were transplanted and thinned to six plants per jar, and thereafter only one-half of the jars were given nutrient solution containing potassium. Macrochemical and microchemical analyses were made of the plants at time of transplanting, flowering stage, and early fruiting period. Total and water-soluble potassium and nitrogen were determined on the base and top portions of the stem and leaves. Total percentages of sugars, dextrin, starch, and hemicellulose were also determined on these same plants.

All samples for analysis were gathered early in the morning. Fractions of the plants, tops and bottom portions, were then taken for total potassium, nitrogen, and moisture determination. Another portion was used for the determination of soluble nitrogen. These

extracts were made immediately. The method used in making the extractions was essentially the same as that described by MacGillivray (*19*), Leukel (*17*), and Janssen (*14*). In these experiments, however, 50 grams of plant tissue were mascerated and then extracted with approximately 1 liter of distilled water. Two hundred cubic centimeters duplicate fractions were used for making the various nitrogen fractionations. Duplicate 100 c. c. portions were used also for soluble potash determinations. A fractional part of the whole sample was used for carbohydrate analyses. This sample was fixed and preserved in 90 per cent boiling alcohol to which a very small amount of $CaCO_3$ was added to neutralize any of the organic acids present. The carbohydrate determinations were made after the method used by Leukel (*17*) or Janssen (*14*). Total sugars, however, were determined by hydrolyzing with 2.5 per cent hydrochloric acid at room temperature for 24 hours. This method seemed to give more consistent results without the loss of sugars and proved better than hydrolysis for 45 minutes on a water bath. A modification of the Munson and Walker method was used for determining the reducing power of sugars, the amount of copper reduced being determined by the Shaffer and Hartmann (*26*) titration method. The percentage of sugars and other carbohydrate forms were always expressed in terms of glucose, the copper equivalents being taken from the Munson and Walker tables.

RESULTS OF EXPERIMENTS

Several preliminary experiments on tomatoes, the detailed results of which are not reported here, were carried out in 1927. In these experiments Marglobe tomatoes were started in silica sand and when the plants were 2 inches high they were transferred to water culture solutions. From the result of this preliminary work on various nutrient solutions, including Knop's, it was found that the plants showed less tendency to become chlorotic when the hydrogen-ion concentration was kept in the vicinity of pH 5 to 6 than if kept at higher pH values. The substitution of $(NH_4)_2SO_4$ for $Ca(NO_3)_2$ as a source of part of the nitrogen in the solution tended to keep the reaction slightly acid. It was partly for this reason that $(NH_4)_2SO_4$ was added to Knop's nutrient solution, which was eventually used in the work. It should also be mentioned that a nutrient solution was desired which did not contain sodium, since it is believed that the ion could function in the plant similarly to the potassium ion. Hence the modified form of Knop's solution was used. A discussion of this is brought out in another paper.[3]

EXPERIMENT 1

To study the effect of potassium starvation together with potassium translocation and the relation of potassium to carbohydrate and nitrogen assimilation, tomato plants were grown on a modified Knop's nutrient solution. (See methods.) A variety of Norton wilt-resistant tomato was planted in sand containing a trace of insoluble potassium salt. The plants were given the basic nutrient solution including potassium. On March 5, when the plants were 6 inches high, one half

[3] BARTHOLOMEW, R. P., and JANSSEN, G. THE RELATION BETWEEN CONCENTRATIONS OF POTASSIUM IN CULTURE SOLUTIONS AND OPTIMUM PLANT GROWTH. Soil Sci. 27: 1929.

of them were transplanted to a solution containing no potassium and the other half to a full potassium solution. At this time 164 plants were harvested, separated into stems and leaves, and analyzed. On March 27 another set of plants was harvested from jars receiving potassium as well as from those containing no potassium in the nutrient solution. This time of sampling is called the bloom stage. The plants at this time were divided into base stem, top stem, and leaf. The third analysis was made some time after blooming, namely, May 4. Very few fruits had set on the plant at this time. The plants were fractionated as follows: Base and top stem; base and top leaves. The division of base and top was arbitrarily made at about the middle of the plant.

TABLE 1.—*A comparison of the total dry weight and percentage dry weight of tomato plants grown on a full potassium nutrient solution the entire growing period with plants receiving no potassium after attaining a height of 6 inches*

FIRST ANALYSIS; 164 PLANTS 6 INCHES HIGH

Leaves		Stem	
Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants
Per cent	*Grams*	*Per cent*	*Grams*
10.25	0.676	9.9	0.658

SECOND ANALYSIS; BLOOM STAGE

Treatment	Number of plants	Base stem		Top stem		Leaf	
		Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants
		Per cent	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*
Low K	42	9.0	3.43	7.1	2.33	13.3	12.02
High K	96	5.9	1.76	5.0	1.074	10.0	6.9

THIRD ANALYSIS; FRUITING STAGE

Treatment	Number of plants	Stem				Leaf			
		Base		Top		Base		Top	
		Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants
		Per cent	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*
Low K	39	15.2	8.78	13.0	9.1	20.5	13.2	15.5	12.31
High K	54	11.3	11.24	10.1	9.16	14.1	15.32	13.2	18.33

The percentage dry weights of the various portions of the plant as well as the actual weights per 10 plants are given in Table 1. It will be noted that at the time of the second analysis the low-potassium

plants were heavier than the high-potassium plants. This was not a natural condition. It may have come about through the fact that at the time of transplanting somewhat larger plants were set in the low-potassium jars, and that during the early development of the plants they were not greatly handicapped by lack of potassium in the solution. During the next period of development, however, the high-potassium plants greatly exceeded the low-potassium plants in growth. This is quite evident in all parts of the plant. As shown in Table 1 the percentage of dry matter is greater in the low-potassium plants than in the high-potassium plants. The significance of this will be touched on later.

TABLE 2.—*Nitrogen distribution in tomato plants grown on a high-potassium nutrient solution*

[First series]

Date analyzed	Stage of development	Part of plant	Dry weight	Total nitrogen	Soluble nitrogen	Filtrate nitrogen	Protein nitrogen	Total potassium
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mar. 5	6 inches high	Leaf	10.25	4.44	1.47	0.846	0.88	2.99
Do	do	Stem	9.9	1.88	1.021	.931	.167	4.07
Mar. 27	Bloom	Leaf	10.25	3.94	.96	.74	.36	2.54
Do	do	Top stem	5.9	2.88	1.58	1.25	.40	6.69
Do	do	Base stem	5.9	2.38	1.61	1.38	.28	6.45
May 4	Fruiting	Top leaf	13.2	3.36	1.64	1.09	.53	3.03
Do	do	Base leaf	14.1	2.78	1.42	1.06	.46	2.85
Do	do	Top stem	10.1	2.60	2.22	1.75	.663	4.30
Do	do	Base stem	11.3	2.60	1.91	1.42	.504	3.58

TABLE 3.—*Nitrogen distribution in tomato plants grown on a full potassium nutrient solution only until plants were 6 inches high*

[First series]

Date analyzed	Stage of development	Part of plant	Dry matter	Total nitrogen	Soluble nitrogen	Filtrate nitrogen	Protein nitrogen	Total potassium
			Per cent	*Per cent*	*Per cnet*	*Per cent*	*Per cent*	*Per cent*
Mar. 5[a]	6 inches high	Leaf	10.25	4.44	1.47	0.846	0.88	2.99
Do	do	Stem	9.9	1.88	1.021	.931	.167	4.07
Mar. 27	Bloom	Leaf	13.3	4.28	1.00	.654	.49	.94
Do	do	Top stem	7.1	2.66	1.91	1.50	.422	1.76
Do	do	Base stem	9.0	2.16	1.43	1.33	.188	1.04
May 4	Fruiting	Top leaf	15.5	4.60	1.93	1.35	.516	.80
Do	do	Base leaf	20.5	4.00	1.71	1.39	.41	.54
Do	do	Top stem	13.0	3.48	2.20	1.77	.454	.48
Do	do	Base stem	15.2	2.48	1.69	1.28	.368	.35

[a] Grown on a high potassium nutrient solution until plants were 6 inches high.

The results for total nitrogen and for total soluble, protein, and filtrate nitrogen, after coagulation by heat, are given in Tables 2 and 3. A direct comparison can be made of the nitrogenous fractions found. The outstanding point in these data is the fact that the total nitrogen and total water-soluble notrogen are higher in the leaf portion of the low-potassium plants than in that of the high-potassium plants. The reverse seems to hold for the stems. These results should be compared with the nitrogen analysis obtained in the second series of plants. (See Tables 9 and 10.)

TABLE 4.—*Carbohydrate distribution in tomato plants grown on a full potassium nutrient solution*

[First series]

Date analyzed	Stage of growth	Part of plant	Dry weight of 10 plants	Dry weight	Reducing sugars	Total sugars	Dextrin	Starch	Hemicellulose
			Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mar. 5	6 inches high	Leaf	0.676	10.25	3.39	3.86	0.326	3.00	4.49
Do	do	Stem	.658	9.9	.752	.88	.326	.48	6.437
Mar. 27	Bloom	Leaf	6.9	10.25	3.94	3.80	.604	5.21	7.93
Do	do	Top stem	1.094	5.9	2.884	5.738	.612	1.080	5.594
Do	do	Base stem	1.76	5.9	2.470	2.862	.506	.905	8.75
May 4	Fruiting	Top leaf	18.33	13.2	10.523	11.121	1.271	2.672	6.898
Do	do	Bottom leaf	15.32	14.1	7.567	8.78	.765	2.0723	6.435
Do	do	Top stem	9.16	10.1	4.662	5.762	1.537	2.218	8.312
Do	do	Bottom stem	11.21	11.3	5.370	6.385	1.431	2.400	8.844

TABLE 5.—*Carbohydrate distribution in tomato plants grown on a full potassium nutrient solution only until the plants were 6 inches high*

[First series]

Date analyzed	Stage of development	Part of plant	Dry weight of 10 plants	Dry weight	Reducing sugars	Total sugars	Dextrin	Starch	Hemicellulose
			Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mar. 5[a]	6 inches high	Leaf	0.676	10.25	3.39	3.86	3.26	3.00	4.49
Do.[a]	do	Stem	.658	9.9	.752	.88	.326	.48	6.432
Mar. 27	Bloom	Leaf	12.02	13.3	4.29	4.23	5.11	6.15	8.29
Do	do	Top stem	2.33	7.1	10.56	11.98	.722	1.95	7.79
Do	do	Base stem	3.43	9.0	8.104	9.263	.610	1.926	9.117
May 5	Fruiting	Top leaf	12.31	15.5	9.632	10.870	1.433	2.870	6.283
Do	do	Base leaf	13.2	20.5	8.64	8.949	1.272	1.543	5.981
Do	do	Top stem	9.1	13.0	4.399	5.705	1.934	1.658	9.914
Do	do	Base stem	8.78	15.2	4.942	5.280	2.507	2.776	9.851

[a] Grown on high nutrient solution till plants were 6 inches high.

Data showing the distribution of carbohydrates in high-potassium and low-potassium plants are given in Tables 4 and 5. In this series the percentage of sugar, in the case of plants in the bloom stage, is greatest in the low-potassium plants. The percentage of starch fluctuates considerably, but except in plants of the bloom stage the highest percentage is also located in plants grown on low-potash solutions. The hemicellulose, except in the leaves at the fruiting stage, is greatest in the low-potassium plants. In nearly all instances the base stems have a higher percentage of hemicellulose than the top stems.

The percentages of potassium in the plants of the first series are given in Tables 6 and 7. These tables show that the high-potassium plants contain much more potassium than the low-potassium plants. It will also be noted that the water-soluble portion includes nearly all the potassium of the plant. In other words, practically all the potassium may be removed from the plant through successive washing with water. The highest potassium concentration is located in the growing portions of the plant.

EXPERIMENT 2

A second series of plants was started on June 27. These plants were treated in exactly the same manner as those of the first series except that they were allowed to grow to a height of 8 inches before they were transplanted to jars treated with a low-potassium nutrient solution. The nutrient solution added to these plants was changed so that a greater percentage of the nitrogen appeared as ammoniacal nitrogen. The reason for this was mentioned previously, namely, that it aids in maintaining a sufficiently acid reaction to keep the iron and phosphate in solution.

TABLE 6.—*Distribution of total and water-soluble potassium in tomato plants grown on a full potassium nutrient solution*

Stage of growth	Number of plants	Part of plant	Oven-dry weight of 10 plants	Water-soluble potassium	Total potassium
			Grams	*Per cent*	*Per cent*
6 inches high	164	Leaves	0.676	2.93	2.99
Do	164	Stem	.658	4.29	4.07
Blossom	96	Leaves	6.9	3.31	2.54
Do	96	Top stem	1.074	[a] 8.53	6.69
Do	96	Base stem	1.76	[a] 7.22	6.45
Fruiting	54	Top leaves	18.33		3.03
Do	54	Bottom leaves	15.32		2.85
Do	54	Top stem	9.16		4.30
Do	54	Bottom stem	11.21		3.58

[a] Differences probably due to different sand used in grinding the plant tissue.

TABLE 7.—*Distribution of total and water-soluble potassium in tomato plants grown on a full potassium nutrient solution only until the plants were 6 inches high*

Stage of growth	Number of plants	Part of plant	Oven-dry weight of 10 plants	Water-soluble potassium	Total potassium
			Grams	*Per cent*	*Per cent*
6 inches high [a]	164	Leaf	0.676	2.93	2.99
Do	164	Stem	.658	4.29	4.07
Blossom	42	Leaves	12.02	.75	.94
Do	42	Top stem	2.33	1.84	1.76
Do	42	Base stem	3.43	1.13	1.04
Fruiting	39	Top leaves	12.31		.80
Do	39	Base leaves	13.2		.54
Do	39	Top stem	9.1		.48
Do	39	Base stem	8.78		.35

[a] Grown on a full potassium nutrient solution till plants were 6 inches high.

Table 8 gives the percentage of dry weights for the various fractions of the plants, for three periods of analysis, namely, at time of transplanting, at blooming, and at fruiting. In general, the relationship between the percentage of the dry weights for the high and low potassium plants is similar to that for the corresponding data obtained in the various analyses of the first series. The low-potassium plants have a higher percentage of dry matter than the high-potassium plants. It should be noted that the high-potassium plants in this series, analyzed at the blooming stage, were heavier on the 10-plant basis than the low-potassium plants. This differs from the result obtained in the first series.

TABLE 8.—*A comparison of the total dry weight and percentage dry weight of tomato plants grown on a full potassium nutrient solution the entire growing period with those of plants receiving no potassium after attaining a height of 8 inches*

[Second series]

FIRST ANALYSIS; PLANTS 8 INCHES HIGH

Number of plants	Leaves		Stem	
	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants
	Per cent	*Grams*	*Per cent*	*Grams*
94	9.0	3.25	5.09	1.94

SECOND ANALYSIS; FULL BLOOM

Treatment	Number of plants	Top stem		Base stem		Top leaf		Base leaf	
		Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants	Dry matter	Oven-dry weight of 10 plants
		Per cent	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*
Low K	62	5.4	1.25	7.6	2.58	9.5	2.95	9.4	4.23
High K	75	5.2	1.865	7.4	4.47	10.6	4.31	7.9	5.12

THIRD ANALYSIS; FRUITING STAGE

Low K	37	7.7	4.19	9.2	6.39	12.3	10.25	12.6	8.67
High K	39	7.1	11.1	9.1	15.1	9.5	25.0	8.5	23.40

TABLE 9.—*Nitrogen distribution in tomato plants grown on a high-potassium nutrient solution*

[Second series]

Date analyzed	Stage of development	Part of plant	Dry weight	Total nitrogen	Soluble nitrogen	Filtrate nitrogen	Protein nitrogen	Total potassium
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
June 22	8 inches high	Leaf	9.00	4.60	0.969	0.647	0.32	2.91
Do	do	Stem	5.09	2.92	1.00	.88	.148	7.43
July 13	Bloom	Top leaf	10.6	4.97	1.36	1.12	.23	3.24
Do	do	Base leaf	7.9	3.94	1.36	1.22	.27	3.46
Do	do	Top stem	5.2	3.52	2.15	2.10	.15	7.90
Do	do	Base stem	7.4	2.00	1.65	1.52	.067	5.47
Aug. 3	Fruiting	Top leaf	9.5	4.94	1.305	1.147	.084	4.03
Do	do	Base leaf	8.5	3.60	1.40	1.21	.058	4.76
Do	do	Top stem	7.1	2.16	1.704	1.478	.141	6.09
Do	do	Base stem	9.1	1.96	1.494	1.494	.0439	4.56

Tables 9 and 10 contain the results on nitrogen distribution from the second series of plants. The increase of total nitrogen as well as the total soluble nitrogen of the low-potassium plants over that of the high-potassium plants is even more pronounced in this series than in the first series. The growing parts of the plant are somewhat high in these compounds.

TABLE 10.—*Nitrogen distribution in tomato plants grown on a full potassium nutrient solution only until 8 inches high*

[Second series]

Date analyzed	Stage of development	Part of plant	Dry weight	Total nitrogen	Soluble nitrogen	Filtrate nitrogen	Protein nitrogen	Total potassium
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 22 [a]	8 inches high	Leaf	9.00	4.60	0.969	0.647	0.32	2.91
Do. [a]	do	Stem	5.09	2.92	1.00	.88	.148	7.43
July 13	Bloom	Top leaf	9.5	6.80	2.90	2.61	.32	1.64
Do	do	Base leaf	9.4	5.00	2.43	2.22	.276	1.3
Do	do	Top stem	5.4	5.00	4.04	3.87	.24	3.68
Do	do	Base stem	7.6	3.52	2.84	2.82	.13	3.31
Aug. 3	Fruiting	Top leaf	12.3	6.36	3.009	2.407	.359	1.66
Do	do	Base leaf	12.6	5.64	2.722	2.507	.2857	1.18
Do	do	Top stem	7.7	4.52	3.76	3.63	.12	2.02
Do	do	Base stem	9.2	4.12	3.72	3.53	.239	1.49

[a] Grown on a high potassium nutrient solution until 8 inches high.

TABLE 11.—*Carbohydrate distribution in tomato plants grown on full potassium nutrient solution*

[Second series]

Date analyzed	Stage of development	Part of plant	Dry weight of 10 plants	Dry weight	Reducing sugars	Total sugars	Dextrin	Starch	Hemicellulose
			Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
June 27	8 inches high	Leaf	3.25	9.0	1.295	1.507	0.493	2.034	5.717
Do	do	Stem	1.94	5.09	1.247	1.425	.624	1.620	8.668
July 12	Bloom	Top leaf	4.31	11.1	2.877	3.406	.582	.530	7.377
Do	do	Base leaf	5.12	7.9	.689	1.176	.456	.228	6.50
Do	do	Top stem	1.865	6.1	1.380	3.649	.764	.690	7.762
Do	do	Base stem	4.47	7.9	1.230	2.382	.560	.651	11.307
Aug. 3	Fruiting	Top leaf	2.340	9.6	5.886	7.307	.756	1.162	9.012
Do	do	Base leaf	25.0	8.6	2.123	2.285	.622	1.015	5.832
Do	do	Top stem	11.1	7.3	5.886	7.397	.756	1.662	9.012
Do	do	Base stem	15.1	9.2	3.748	5.481	.704	.808	10.733

TABLE 12.—*Carbohydrate distribution in tomato plants grown on a full potassium nutrient solution only until plants were 8 inches high*

[Second series]

Date analyzed	Stage of development	Part of plant	Dry weight of 10 plants	Dry weight	Reducing sugars	Total sugars	Dextrin	Starch	Hemicellulose
			Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
June 27 [a]	8 inches high	Leaf	3.25	9.0	1.295	1.507	0.493	2.034	5.717
Do. [a]	do	Stem	1.94	5.09	1.247	1.425	.624	1.620	8.668
July 12	Bloom	Top leaf	2.95	10.1	1.115	1.800	.549	.950	5.801
Do	do	Base leaf	4.23	10.0	1.257	1.651	.702	.826	6.681
Do	do	Top leaf	1.25	5.4	1.456	2.373	.685	1.218	8.528
Do	do	Base leaf	2.58	8.1	1.508	1.914	.728	.491	10.315
Aug. 3	Fruiting	Top leaf	10.25	12.5	3.890	4.403	.670	1.051	5.670
Do	do	Base leaf	8.67	12.6	2.870	3.406	.687	.812	5.490
Do	do	Top stem	4.19	8.0	2.120	3.036	.585	.551	8.965
Do	do	Base stem	6.39	9.3	2.344	2.956	.505	.532	10.688

[a] Grown on high potassium nutrient solution till plant was 8 inches high.

The carbohydrate data for high and low potassium plants are given in Tables 11 and 12. The consistently high percentage of total sugars is not noted for the low-potassium plants as it was in the previous series of analyses. It is apparent that from the data at hand very few deductions can be made as to the relation of potassium to total sugars in these plants. In the previous series of analyses the carbo-

hydrate percentages were higher in the low-potassium plants at time of blooming than in the high-potassium plants. At this early analysis the tops were higher in these carbohydrate compounds than the base. At a later analysis, designated as fruiting, the percentages of carbohydrates in the base of the stem were about the same as those in the top of the stems. Also in the last analysis of the first series, the total percentage of carbohydrate compounds were approximately the same in the stems of the high-potassium plants as in those of the low-potassium plants. A study of the results of Tables 11 and 12 for this last series reveals that the effect of potash starvation upon carbohydrate formation took place much earlier in the development of the plants than it did in plants of the preceding experiment. In this series, in contrast to the results of the first series, the high-potassium plants at the blossom stage, with the exception of the base leaves, contained more carbohydrate compounds than the low-potassium plants. At time of fruiting this condition has become intensified. The high-potassium plants seem in most cases to have a higher percentage of sugars in the top stems than the low-potassium plants. This is particularly noticeable after long potassium starvation.

TABLE 13.—*Distribution of total and water-soluble potassium in tomato plants grown on a full potassium nutrient solution*

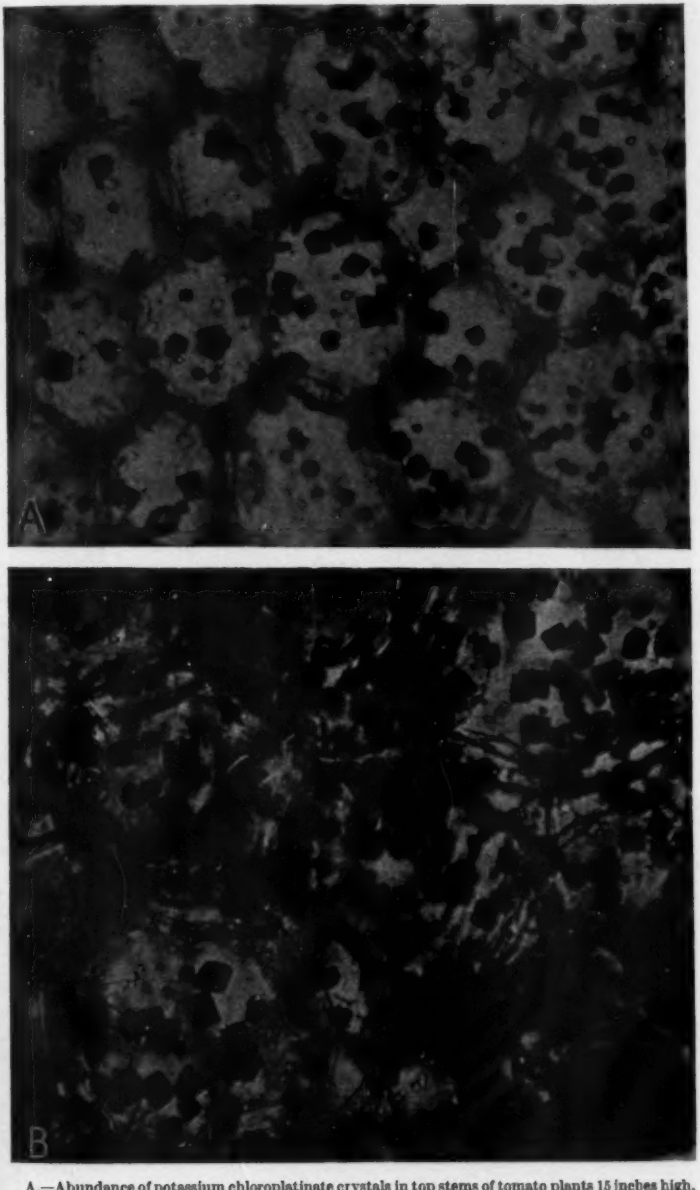
Stage of growth	Number of plants analyzed	Part of plant	Oven dry weight of 10 plants	Water-soluble potassium	Total potassium
			Grams	*Per cent*	*Per cent*
8 inches high	94	Leaves	3.25	3.24	2.91
Do	94	Stems	1.94	6.30	7.43
Bloom	75	Top leaves	4.31	3.67	3.24
Do	75	Base leaves	5.12	4.09	3.46
Do	75	Top stems	1.865	[a] 9.35	7.90
Do	75	Base stems	4.47	[a] 7.00	5.47
Fruiting	39	Top leaves	23.40	4.43	4.03
Do	39	Base leaves	25.0	4.79	4.76
Do	39	Top stem	11.1	6.35	6.09
Do	39	Base stem	15.1	4.63	4.56
Do	39	Roots			1.44

[a] Difference probably due to different sand used in grinding plant tissue.

TABLE 14.—*Distribution of total and water-soluble potassium in tomato plants grown on a full nutrient solution only until plants were 8 inches high*

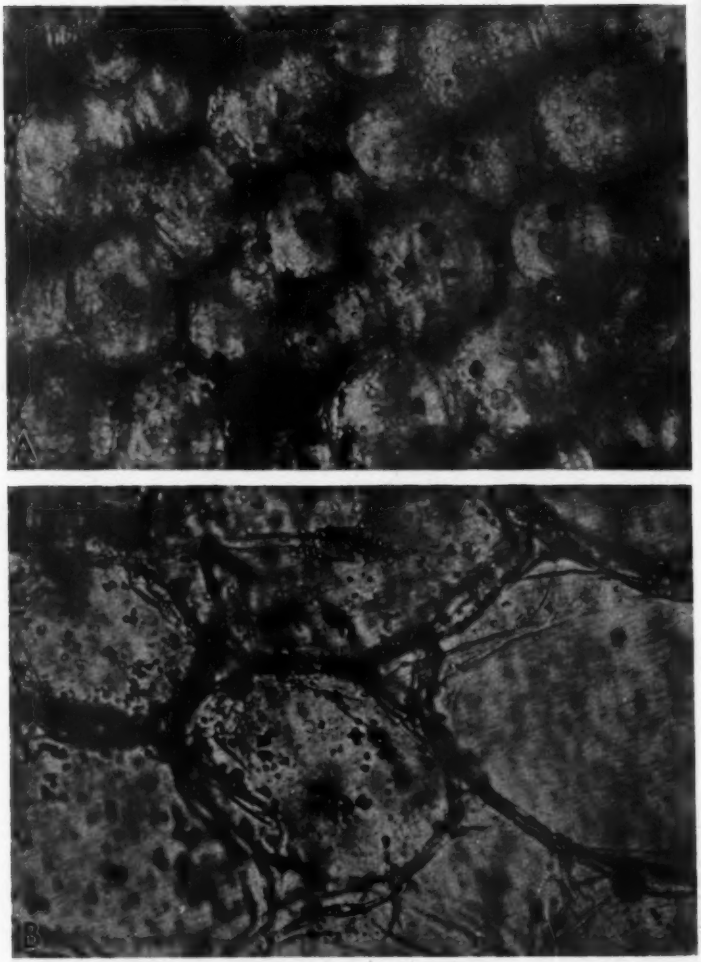
Stage of growth	Number of plants analyzed	Part of plant	Oven dry weight of 10 plants	Water-soluble potassium	Total potassium
			Grams	*Per cent*	*Per cent*
8 inches high [a]	94	Leaves	3.25	3.24	2.91
Do.[a]	94	Stems	1.94	6.30	7.43
Blossom	62	Top leaves	2.95	1.79	1.64
Do	62	Base leaves	4.23	1.07	1.31
Do	62	Top stem	1.25	3.65	3.68
Do	62	Base stem	2.58	2.47	3.31
Late blossom		Base dead leaves			.38
Fruiting	37	Top leaves	10.25	1.31	1.66
Do	37	Base leaves	8.67	.94	1.18
Do	37	Top stems	4.19	1.75	2.02
Do	37	Base stems	6.39	1.48	1.49
Do	37	Roots			.30

[a] Grown on a full potassium nutrient solution till plants were 8 inches high.



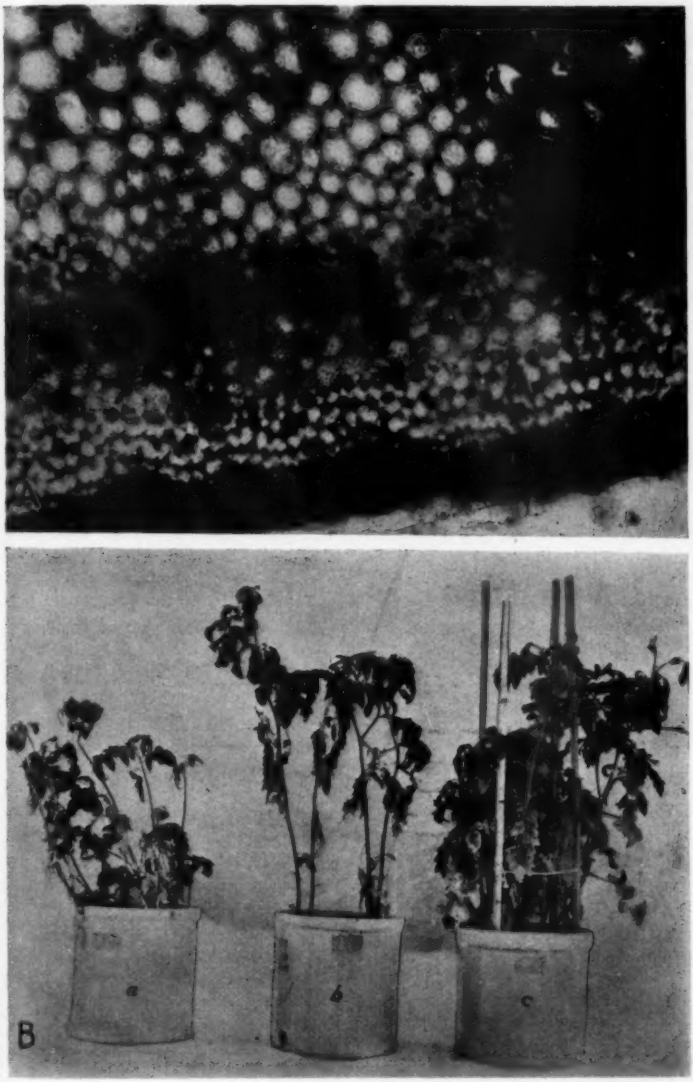
A.—Abundance of potassium chloroplatinate crystals in top stems of tomato plants 15 inches high, grown in sand receiving complete nutrient solution.

B.—Potassium chloroplatinate crystals in base stems of tomato plants 15 inches high, grown in sand receiving complete nutrient solutions.



A.—Scarcity of chloroplatinate crystals in top stems of tomato plants 15 inches high, grown in quartz sand receiving potassium deficient nutrient solution.

B.—Absence of potassium chloroplatinate crystals in base stems of tomato plants 15 inches high, grown in sand receiving potassium-deficient solution.



A.—Translocation of potassium to cambium region in plants grown in potassium-deficient cultures.

B.—Effect of potassium deficiency on growth of tomatoes: *a*, Small amount of potassium supplied at planting; *b*, no potassium added after transplanting; *c*, complete nutrient solution.

The results of the potassium analyses for this second series of plants are given in Tables 13 and 14. The data are essentially the same as in the preceding experiment. The high-potassium plants contain on an average two or three times as much potassium as the low-potassium plants. It should also be noted that the highest percentage of potassium in the low-potassium plants is always found in the upper portions of the stem; that is, in the top leaves and top stem. This is similar to the results obtained in the analysis of plants of the first series.

To study the minimum potassium requirement for the tomato plant, a series of plants was started in a water-culture solution similar to that mentioned previously for preliminary work. The potassium was added in the following concentrations: 0, 0.5, 1, 2, 3, 5, and 10 parts per million. Norton wilt-resistant tomatoes were transplanted to the solution culture when the plants were 2 inches high. After a few weeks of growth in these solutions, the plants were analyzed microchemically for potassium by precipitating the potassium with platinic chloride in an alcoholic solution. The analyses were made on various parts of the plant, particularly the upper and basal regions. The analysis showed that there were no potassium chloroplatinate crystals in the base and few or none in the upper portion of the stems of the no-potassium plants. The 0.5 parts per million plants contained very few potassium chloroplatinate crystals in the basal stems, with a few more at the top. The plants grown on the 10 parts per million solution had as many crystals, according to estimate, as the plants grown in a 25 parts per million potassium solution.

These findings led to an intensive microchemical analysis of the potassium in tomato plants which were used in translocation studies. These studies again substantiated the previous findings, namely, that in low-potassium plants very few or no potassium chloroplatinate crystals were present in the basal portions of the stem; more crystals, however, were present in the growing region. The potassium chloroplatinate crystals in the low-potassium plants were confined largely to the pith, cambium, and very top portions of the stem. These analyses were made at blossoming time, some 30 days after transplanting, and again at the fruiting stage. Both stages were relatively alike except that the potassium chloroplatinate crystals were fewer at the last analysis. The high-potassium plants showed an abundance of potassium chloroplatinate crystals in all portions of the plants. Microphotographs were taken of free-hand sections of basal and top stems of high and low potassium plants. These are presented in Plates 1, A and B, and 2, A and B. In some free-hand sections made of the base stem of plants which showed severe potassium starvation very few potassium chloroplatinate crystals were found. These crystals were largely localized in the cambium region. When this occurred the pith of the plant was entirely free of potassium. (Pl. 3, A.) These findings are in close accord with those of Dowding (*1*), who worked with *Picea canadensis*. These results suggest that the potassium ion may be essential for cell division.

Mention should be made of the symptoms which accompany potassium starvation in the tomato. When potassium starvation occurs in the plant there is a retardation in growth rate. The leaves may take on a dark-green color and become wrinkled. The stems

are streaked with a brownish purple color. There is a tendency for the growth of the stem to continue in length, but the diameter will remain approximately the same. The lower leaves later wither in large numbers. They first turn grayish green, and then dry up without falling off the stem. (Pl. 3, B.) As these leaves die off new ones are formed above. Potassium analyses were made on these old leaves. The results show (Table 14) that they contain very little potassium, namely, 0.38 per cent, in contrast to 0.54 to 0.80 per cent in leaves that remained green and attached to the stem. These results are somewhat similar to those obtained by Smith (*28*). Working with wheat, corn, and buckwheat, he found, as the result of potassium starvation, a dwarfing of the axis and a progressive death of the foliage, the older leaves succumbing first. These latter symptoms are very similar to those obtained by the writers on the tomato studies. Additional evidence to substantiate the potassium translocation data was obtained by determining the percentage of potassium in the roots of the two types of plants at the time of last analysis. These data show that roots of the high-potassium plants contain 1.44 per cent potassium, whereas the roots of the low-potassium plants contain 0.30 per cent potassium, indicating that nearly all the residual potassium had been translocated. By multiplying the total dry weight per 10 plants by the percentage of potassium in each series of analysis, it is found that the amount of potassium in the low-potassium plants after the first analysis remains about the same. This is particularly evident in the second series, in plants analyzed at 6 inches in height and again at blooming stage. Here the amounts of potassium of the two series are identical. At fruiting stage the total amount has increased slightly.

DISCUSSION

The fact that potassium is universally abundant in plant tissues, as noted by data given in Wolff's tables (*31*), makes it evident that it has an important function to perform in the plant. Various interpretations of the function of potassium in the plant have been reported. Loew (*18*, p. *28*) suggests that in green plants potassium is concerned not only in the synthesis of carbohydrates but also in the synthesis of the protein bodies. Thatcher (*29*, *p. 8*) suggests that the "photosynthesis of starch or the changes necessary to its translocation (it is not yet certain which) is so dependent upon the presence of potassium in the cell sap that the whole process stops at once if an insufficient supply is present." It appears, therefore, that potassium must act in some form as an agent in the production or translocation of the carbohydrate compounds. That some investigators have obtained negative correlations between potassium fertilization and carbohydrate accumulation is indicated by more recent investigation. These investigations have usually been conducted under soil-plot conditions, and therefore can not be definitely interpreted. Morse (*10*, *p. 7a*) at the Massachusetts station found that sugars were consistently lower in the wood of plants grown on soil treated with potassium chloride. He found, however, that the starch and pentosans were not so consistent. Nolte (*22*) found that potatoes when grown on weak diluvial sand soil with siliceous subsoil, which was treated with potassium magnesium sulphate and

potassium chloride, had a lower starch content. Jacob (*13*) found that when potassium magnesium sulphate was applied to moor soils it tended to increase the starch content but not the total yield. Dusserre (*2*) reports data from a Chasselas vineyard located on a gravelly, calcareous soil of France, showing that potassium fertilizers significantly increased the grape yields as well as the sugar content. Tottingham (*30*) grew wheat plants in constant temperature chambers on a nutrient solution containing the full potassium solution, a 0.5 potassium solution, and a 0.2 potassium solution. For his first series of plants grown at a temperature of 25°, he obtained the largest percentage of polysaccharides when the plants were grown on the 0.2 potassium culture solution, but at a lower temperature, 13°, the full and 0.5 potassium culture solutions favored the highest polysaccharide formation. Hall (*8, p. 105*) reports work from the Rothamsted station, where they found that the addition of 500 pounds of potassium sulphate to a combination of ammonium sulphate rape cake and superphosphate increased the yield of mangold roots as high as 37.5 tons per acre.

On the surface, the results obtained on the carbohydrate analysis reported here seem somewhat conflicting. The analyses of the two experiments reported vary considerably in actual percentages of the various compounds in the plant. This may be accounted for partly by the change in the nutrient solution. A close study, however, shows considerable similarity within the two series of analyses.

The percentage dry weight for the two types of plants (high potassium and low potassium) is specific. It is apparent that in this experiment an abundance of potassium in the nutrient solution tended to produce a succulent type of plant growth. This, together with the fact that the weight per plant is much greater for the high-potassium plants, as noted in Tables 6, 7, 13, and 14, shows that it is instrumental in producing rapid growth. Hornberger (*12*) suggests that potassium salts may not always increase grain yields, but that the effect is frequently manifested in the increased yield of straw. The effect of potassium on the dry matter of the plant seems to be very similar to that produced by phosphorus. MacGillivray (*19*) has shown that an insufficient amount of phosphorus in the nutrient solution tends to treble the percentage dry weight of the tomato plant in two months. Such a pronounced difference was not noted in the percentage of dry weight with potassium, but certainly the differences are definite.

The data on carbohydrate assimilation in high and low potassium plants seems to indicate that up to a certain concentration the potassium ion aids carbon assimilation. If, on the other hand, the concentration of potassium is increased the carbon assimilation is again decreased. In the first series of experiments a large difference between the carbohydrate content of high and low potassium plants was noted. This was true primarily of those plants harvested at the bloom stage, or approximately 26 days after transplanting. It may have been that the potassium which was absorbed by the plant up to the time that it reached 6 inches in height was sufficient to take care of its normal metabolic processes; however, 39 days after blooming, or after the second analysis, the greater abundance of carbohydrate was found in the high-potassium plants. This was particularly true of the top leaves and base stems. The base leaves of the

low-potassium plants at that time still had the highest percentage of carbohydrate substances. Similar conditions seem to have prevailed in the second series (see Tables 11 and 12), although the percentage of carbohydrate compounds in the plants of this series was never so great as in the plants of the first series. In the second series also the plants at fruiting stage, 42 days after transplanting, showed the greatest amount of carbohydrates in the high-potassium group.

It should be remarked that low-potassium plants, particularly in the first series, always, until well after blossoming, had a very dark-green foliage. This was so pronounced that a casual observer could easily pick the low-potassium plants from the high-potassium plants on this basis. In addition to this dark-green color, the low-potassium plants took on early in the blooming period a somewhat characteristic ruffled leaf. This was not unlike the type of leaf described by Owen (*23*) in certain strains of soy beans which stored all the starch and sugars in the leaves and stems. It is highly possible that the sugars found in the leaves of these plants could not be translocated with sufficient rapidity, and wrinkling resulted. This, however, was not substantiated by qualitative chemical tests. The second series of plants never had the pronounced deep-blue color or the intensified wrinkled leaves of the first series. Nevertheless the low-potassium plants did have a darker green color than the high-potassium plants. These symptoms, together with results from the carbohydrate analysis, lead to the belief that no serious potash starvation had taken place at the blooming stage.

The relation of nitrogen to potassium in high and low potassium plants was touched on in the presentation of data. It was noted that the percentage of nitrogen, both water soluble and total, was greater in the low-potassium than in the high-potassium plants. It should also be noted that the total nitrogen for the low-potassium plants was much greater in the first than in the second series of plants. The potassium was also considerably higher in the second series. The nitrogen and potassium therefore seem to bear an inverse relationship, namely, wherever a high percentage of potassium is found in the plants a low percentage of nitrogen is likely to occur. These results on the effect of application of potassium on the potassium-nitrogen relation in the plant are similar to the potassium and nitrogen relationship reported in the work of Emerson (*3*), who worked on the potassium-nitrogen ratio of red clover grown on Miami silt-loam soil to which he applied potassium chloride, K_2SO_4, and kainite (12 per cent K_2O) supplemented with applications of manure, superphosphate, and combinations of both. Godlewski (*5*), however, reported that the ratio of potassium to nitrogen in the tubercles of potatoes was found to be characteristic of an abundance or deficiency of available potassium in the soil, increasing with an abundance and decreasing with a deficiency of available potassium. His findings were based on a 4-year test of crops grown on soil to which potassium was added. The writers' results do not agree with those of Godlewski, and perhaps this should not be expected, since he worked with an underground crop. MacTaggart (*20*), however, like Godlewski (*5*), found that potassium had a tendency to increase the total nitrogen and dry matter in Canadian field peas and alfalfa, but that soy beans showed a decrease in these factors due to potassium. From these results it appears that the amount of available potassium in the soil may cause

the potassium-nitrogen ratio in the plant to vary and that this ratio may differ for different species of plants. Loew (*18*) gives data taken from Wolff's tables (*31*) which show that legume seeds are much richer in potassium than are starch seeds such as cereals. Haas and Hill (*6*) conclude in part from a summary of work of Stoklassa on bacterial and beet cultures "that the action of potassium is partly indirect, and only in darkness does it play an important rôle in the production of proteins."

The data on the potassium taken up by the tomato plant at various stages of development as given in Tables 6 and 7 and 13 and 14 indicate that a large amount of the potassium ion is taken up in the early stages of growth. This appears to be due to the fact that the plant is actively growing and needs a high concentration of potassium in its metabolism, as well as in cell division. This is indicated by the wide differences in the potassium concentration of the young and older regions of the stem. Thus for the first series at the time of first analysis the stems had a potassium concentration of 4.07 per cent. At the second analysis the top stems had a concentration of 6.69 and the bottom stems a concentration of 6.45. At a still later date of analysis the top stems contained a potassium concentration of 4.30 and the base stems of 3.58 per cent. These figures are results from the high-potassium plants. If a study is made of the potassium concentration of the low-potassium plants the differences are even more striking. (See Table 7.) Thus for the blooming stage the top stems contain a potassium concentration of 1.76 per cent and the bottom stems 1.04 per cent; at the fruiting stage the top stems contain a potassium concentration of 0.48 and the bottom stems 0.35 per cent. These results are conclusive, showing that the growing regions are higher in the potassium ion.

It will also be noted from the data in Tables 7 and 14 together with data presented from microchemical analyses, that the potassium ion is extremely labile. Due to this lability, as was also noted by Pfeffer (*25 v. 1, p. 585–586*) and Schimper (*27*), the potassium is moved from one region of the plant to the other as needed. It has already been noted that sufficient potassium was absorbed by the plant in the early stages of growth, to enable it to function normally until it had reached or passed the blooming stage. This uptake of potassium during early growth may have an important bearing on the fertilization of agricultural plants with potassium fertilizers.

It is generally assumed that potassium fertilizers are fixed rather quickly as secondary minerals after they are applied to soils. The plants then absorb the potassium as it is liberated from these compounds. The amounts found in the plant tissue during the early stages of growth would depend upon the rate at which the potassium became available from these secondary minerals.

The experiments reported in this paper suggest that a different process may take place after the application of potassium fertilizers to soils. The young plants may absorb during early stages of growth large quantities of the potassium which have been added to the soil. As the plants develop the potassium is translocated to those parts in which there is a deficiency of potassium. This deficiency may be due to the concentration in the soil solution being lower than the amounts necessary for optimum growth. This idea agrees very well with the facts generally found, that a high concentration of potassium in the

plant tissue during the early stages of growth is followed by a very marked decline as the plant matures. However, this explanation does not exclude the possibility of some potassium being fixed in the soil.

The ability of plants to translocate potassium from older to embryonic regions explains why we do not see evidences of physiological disturbances, due to potassium deficiencies, in plants growing on soils on which crops are known to respond very greatly to applications of potassium fertilizers.

The above results on the early uptake of potassium are similar to those obtained by Haley (*7*), who found that buckwheat seedlings have a great avidity for potassium. Pember (*24*) studied the economical use of nitrogen, phosphorus, and potassium on small grain seedlings grown in nutrient solution. He found that the seedlings may absorb potassium early in the development of the plant. It was concluded that wheat was able to make more effective use of a limited amount of potassium if supplied during a 4-week period (from the third to the seventh week from the seed) than if the same amount was applied over a period of 12 weeks. On the other hand, Hartwell (*9*) states that the "feeding power of crops may depend more on their longevity, and on the fact that more active growth occurs at different times than to marked difference in ability to absorb nutrients during one final period under a uniform set of conditions."

Gerricke (*4*), in a study of the influence of temperature on the relation between nutrient salt proportions and early growth of the wheat plant, found that the greatest potassium requirement was obtained at the lower temperatures. Tottingham (*30, p. 317*), however, in studying the effect of temperature on the metabolism of wheat, found that the potassium uptake was greatest at the higher temperature (23° C.) where a full potassium nutrient solution was used, but at a lower plane of potassium supply his results show a greater requirement of the element at a lower temperature (13° C.). The results of these workers indicate that temperature exerts a decided effect on the uptake of potassium salts by the plants when grown under different temperature ranges. Le Clerc and Breazeale (*16*), in studying the translocation of plant food and elaboration of organic plant material in wheat seedlings, found potassium to be taken up early in the development of the plumule. They analyzed the seeds (*16, p. 11*) for potassium, nitrogen, and phosphorus 12 days after germination, and found that after this time 4 per cent of the original potassium, 17 per cent of the nitrogen, and 20 per cent of the original phosphorus remained in the seed. This, together with considerable other data, shows that young wheat plants have a great avidity for the extraction of potassium from cultural solutions. Hopkins (*11*) suggested that the plant may take up more potassium than it needs for normal metabolism, and that this excess is tolerated rather than required.

The results obtained from macrochemical analyses as well as microchemical analyses show that potassium is taken up in large amounts by plants in early growth. Further observation on plants going through a period of potassium starvation with a progressive dying off of the older leaves, which contained very little potassium and the continued formation of new leaves relatively well supplied with potassium, seem to furnish good evidence that the potassium ion is extremely mobile and can be reutilized in the metabolic processes of the plant.

It was apparent when tomato plants were grown on a high potassium solution till they were 6 or 8 inches high and then transplanted to a minus potash solution that they had taken up sufficient potash during the early period to maintain their development up to and including the blooming period. This is evident by the growth rate as well as by the carbohydrate relationship in the plants up to that time. The relatively normal growth, for a period at least, after the plants were transplanted to a minus potassium culture solution would indicate that the potassium in the plant would have to be translocated to the growing regions.

The facts that there never was any extreme difference in the sugar relationship of the high and low potassium plants, even at the fruiting stage, and that there were large amounts of soluble nitrogen present in the low potassium plants, suggest to the writers that a condensation of these substances to the higher carbohydrate and nitrogen compounds may not have been possible without the presence of the potassium ion. Consequently growth was inhibited in later stages of development.

SUMMARY

A study was made of the tomato plant in relation to its carbohydrate, nitrogen, and potash assimilation. The plants used were grown on a full potassium nutrient solution till 6 inches high, and then transplanted to a sand culture containing no potassium. Checks were given the same treatment but kept growing on a high-potassium nutrient solution. The carbohydrate compounds, soluble and total nitrogen and potassium, were determined at different stages of growth by macrochemical analyses. The potassium was also determined by microchemical analyses. Analyses for reducing the total sugars, dextrin, starch, and hemicellulose were made at various stages in the growth of the plant.

1. The results show an increase in the percentage of dry matter in all parts of the low-potassium plants. The total dry weights of high-potassium plants increased rapidly after the bloom stage.

2. The total and soluble nitrogen was much higher for the low-potassium plants. A high percentage of potassium in plants was correlated with a low percentage of nitrogen. There is an inverse relationship between the potassium and nitrogen content of the plant when there is insufficient potassium for normal growth.

3. There seems to be an optimum potassium concentration which is conducive to the normal assimilation of carbohydrate compounds, and above and below which assimilation is reduced. This optimum relation has not been found as yet.

4. In potassium-starved plants the potassium seems to be transferred to and localized in the meristematic and growing portions of the plant. There is evidence to indicate that the potassium ion is necessary for cell division. Practically all the potassium was present in a water-soluble form.

5. Leaf starvation of the tomato, due to a lack of potassium is progressive, extending from the lower to the upper leaves. The dead leaves were relatively free from potassium, indicating that potassium had been translocated and reutilized by the growing portions of the plant.

6. It is suggested that, inasmuch as high nitrogen and high sugars are correlated in the blooming stage of low-potassium plants, the absence of good growth may have been due to a lack of condensation or polymerization of these compounds to more complex forms.

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INDEX